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THE PSYCHOLOGY OF DRAWING BLOOD SPECIMENS FROM CHILDREN*

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Terror is the reaction of about ninety per cent of the children who are brought to the laboratory to have specimens of blood drawn. Small wonder! Through their eyes many have seen a dead-white room with a serious-faced medical technologist (three to four times their sizes—a real monster!) approaching them with a wicked-looking instrument and saying, "I'm going to stick you." Let's face it—we, through stupidity and thoughtlessness, have far too frequently produced conditioned reflexes that have lasted a lifetime.

Our problem consists of two different types of patients—those who have already been conditioned against the bleeding room and those who have not. The first usually enters the laboratory screaming and is not at all averse to kicking, clawing or biting the phlebotomist. The latter is wide-eyed with the strangeness of the event, and will probably soon join the others.

In order to prevent fear it is first essential that we have a more pleasant environment. There is no reason why a bleeding room should be less attractive than a home. Pastel walls with figures from nursery rhymes are sure to have a relaxing effect on the most wary patient. The attention of the children may also be drawn to additions as the balanced aquarium. They become so fascinated with the graceful movements of the fish that they temporarily forget their qualms. Electrically or mechanically powered toys that move while the young patient is present captures his interest immediately and holds it. Figure 1 illustrates

* First A.S.M.T. Award. Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

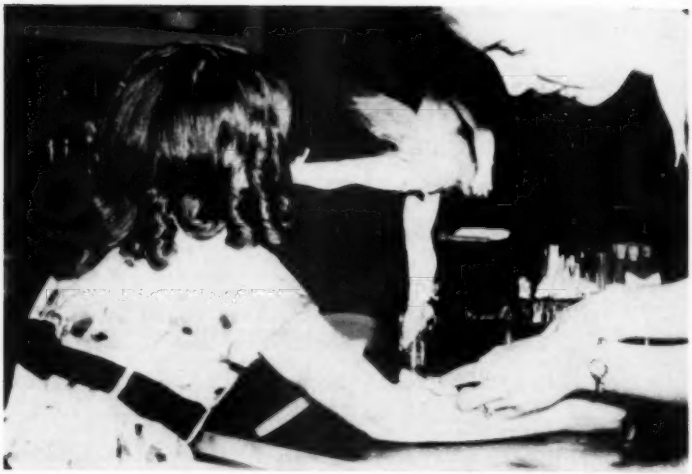

Figure 1. Ballerina doll to distract attention from blood drawing.

a child having her blood drawn while a doll distracts her. The ballerina doll may be mounted on a musical base which revolves, making the doll appear to dance. The motion of the doll, together with the music, keeps the child absorbed and she is much less aware of the drawing of the blood. If unlimited space is available, the furniture should be styled to suit the children. Any cowboy-spirited boy would be thrilled to straddle a horse while having his blood drawn, and a tiny throne would enchant the heart of the most frightened girl. The room, with a little imagination, may be converted into a veritable fairyland. There is practically no way the scientific instruments can be camouflaged, but they can be kept in containers that resemble nursery characters.

One of the best ways to make the patient feel at ease is through music. A jaunty tune such as "Whistle While You Work" from *Snow White and the Seven Dwarfs* automatically bolsters the courage, while keeping the tot's mind preoccupied. The main purpose in treatment such as this is to make a trip to the laboratory so pleasant that it seems like a treat instead of punishment. If the first experience is made to have a happy effect on the child, he will not only look forward to subsequent visits, but will also motivate his friends in the same way. Children like to boast to their playmates of adventures they have experienced, and will do so, even if they do not like the experience. So if the medical

profession works cleverly, the children will become salesmen *for* us instead of *against* us. Ordinarily, children have a motivation for new experiences—this should be used to advantage.

We may well apply the psyhcology which Crane[1] has suggested. A classmate of his gained as much prestige in five years as most New York physicians attain in twenty. And it was chiefly through his magic that he did it, for churches and men's organizations were eager to have him put on his sleight-of-hand show. Children were delighted to visit his office for he would pull rabbits out of their shirts or perform other interesting tricks. So they would talk about him among their friends until other children, as well as their parents, wanted to consult this physician.

Another bit of interesting distraction is the use of zepherin solution frozen into ice cubes which have been colored pink or blue. The melting zepherin sterilizes the skin, and the cold application acts as a mild local anaesthetic, making the skin puncture painless. Children who must have many blood samples drawn are very enthusiastic about this method.

It is quite obvious that the combination of strange objects, situations and persons, together with sudden unexpected movement and pain, produce fear. Stoddard and Wellman[2] reported that the mean number of fears for children was 2.7, the most feared objects in order being the following: dogs, doctors, storms, deep water and darkness. Analysis of the situations revealed that lack of preparation and strangeness of situation were paramount causes, with pain involved in one-third of the cases and restraint in one-fourth. There was a tendency for the child to have fears corresponding to those of his mother, the correlation being 0.67 ± 0.05 between the number of children's fears and the number of the mother's fears. Strang[3] quotes that fear is usually aroused by something that has the element of strangeness, suddenness, or unexpectedness. A frog was found to be the most fear-provoking of the animals presented, probably because of its tendency to jump unexpectedly. Breckenridge and Vincent[4] believe that learning is most permanent when the learning situation is most vivid. Attitudes and emotional conditions set up under emergency or unusual circumstances make a far more permanent impression on children than would be made under less emotional circumstances. However, fear usually fades unless it is sustained by fantasy. Frequently it is nourished by the home or school.

Morgan[5] states that fears may be copied from adults. The mother communicates the fear to the children who imitate her. An adult who had a senseless fear of the dentist's office could trace the origin of this fear to her early childhood. Her mother had a similar dread but would take her daughter with her to

keep her company. The little child would sit in front of her mother, living vicariously through a great amount of imaginary torture along with her jittery mother, who would yell in terror whenever the dentist approached her. It is deemed advisable to ban this type of adult from the room to prevent transferral of fear. Children may also learn that fear demonstrations are effective in influencing elders to conform to their wishes, and this type of ulterior motive should be recognized apart from true apprehension.

Strang[6] believes to conceal one's own fear is difficult if not impossible because of the many subtle, subvocal ways in which feeling may be communicated. Therefore if the parents accompanying the patient are unduly excited or nervous, it is better to ask them to wait in the lobby. Or the technologist may confide that she doesn't like to be stuck with a needle either. This brings the child and technologist closer together in working on their common problem. When a child admits fear, he should be met with understanding. It is easier to reason with a child who will discuss his phobias. Much of the value of the technique of helping the child to cope directly with his fears lies in the fact that in the process the child benefits from the companionship fellow suffering of the adult. It makes him feel that he need not be ashamed of his fear and that he is not alone. Sherman[7] in his study on psychology for nurses found that children usually project their security upon their parents because of their close identification with them. One who feels insecure is more critical of others. The greater the identification of parents with their children, the greater the drive to obtain sympathetic reactions from others. Therefore these children have more difficulty adjusting to others. In this case it is often unwise to have the parents leave the room. Above all, avoid a feeling of isolation. When the mother holds the child in her lap, it provides a sanctuary, making the child feel protected and relaxed.

Diseases should never be discussed in the presence of children, lest they become alarmed that they are afflicted with an incurable malady. Suggestion is a subtle force and it is best to avoid saying anything about that which we do not wish to infer. An example of this is the child who, when told that he was being tested for diabetes, thought he was going to die, because of the resemblance in the sound of the two words.

It is wise to speak on the same level as the child—use monosyllables whenever possible. A child exhibits more confidence in the phlebotomist who explains what he is about to do. Be careful not to overassure—this would make the child suspicious. Never tell the child that a finger-tip puncture or venipuncture is not going to hurt. If you do, he may never trust a technologist again. A soothing intonation of voice is also of basic importance. Chil-

dren are more susceptible to an unpleasant voice than many adults because, not understanding fully the words, they rely on the sounds to relay the moods of others. Even small babies will respond to soothing or harsh tones.

The patient should become accustomed to the technologist as a kindly person before a painful procedure is begun. Jersild[8] has pointed out that fear of strangers frequently spreads to other persons. When such symptoms occur, they may wane within a short time. Voices and faces that were unfamiliar tend, in time, if they recur, to precede into the realm of the familiar. So in cases of extreme fear, it would seem advisable for the technologist to leave the child alone in the room with the mother, and then reappear several times, until the fear partially subsides.

Direct compliments make the young patient know that we are aware that he is more than a network of veins. The younger a child is, the more direct the compliments should be. It is advisable to be specific, such as, "That is a lovely bracelet you are wearing!" or "Where did you get that sharp cowboy tie?"

Most children are blessed with vivid imaginations which can be used to benefit. An extremely obstinate little girl entered the clinic one day to have tests run in connection with her obesity. Her face expressed fear, and she displayed not a spark of cooperation. She was addressed in a calm, soothing manner for a while and as the phlebotomist tied the tourniquet on the arm she confided. "Did you know that many princesses and queens wear bracelets above the elbow where I'm placing this bracelet?" The pressure of the tourniquet was no longer noticed and the young lady turned momentarily into a Cinderella. Playing out the role, the tot behaved in typical majestic fashion, and her fears appeared to be dispelled.

Coaxing is far superior to clubbing, according to Crane.[9] When a patient is unduly uncooperative, sometimes an appeal to his pride will turn the trick. Above all, avoid negative suggestions, as "Don't"—you may be producing ideas that he wouldn't have thought of himself.

It should be remembered that, to a child, everything looks much larger than it does to an adult. Note that in Figure 2 the shortest needle and smallest syringe possible is being used. A child will pay more attention to the length of the needle than he will to the gauge. A large syringe may make an impression that the medical technologist intends to get his last drop of blood.

The child must never feel that the phlebotomist lacks confidence in her work. Efficiency is recognized by lack of hesitancy of any kind. A pause on the part of the tech will probably increase the fear of the child and cause him to be less in agreement.

Age plays an important role in fear. Fears are not inherited—they are learned or conditioned. Four-year-olds are more afraid

Figure 2. Use of a short needle and small syringe to reduce fear.

than two-year-olds. It has also been found that fears wax and wane with changes in the child's basic security and ability to control his environment. His perception of the situation influences his responses. It is interesting to note the report of Jersild[11] illustrated in Figure 3. This describes certain age trends in children's overly expressed fears, notably a decline in fears with age in response to certain tangible and immediate situations, and an increase with age in the percentage of children who show fear of imaginary creatures. Older children are different in response in that they respond to anticipations rather than the immediate environment which stimulates the newborn infant.

In another study, Jersild[11] reported children's own descriptions of their fears and the worst things that ever happened to them. In describing "worst happenings," 72.7% of the children interviewed listed bodily injury, falling, illness, traffic accidents, operations, hurts and pains, etc. Their fears are also affected as they grow older and the fears become increasingly concerned with imaginary dangers.

The body goes through many changes during strong emotional responses, such as fear (Kelly).[12] These include changes in breathing, in circulation of the blood, and in digestion. Fear and anxiety have been listed as probably causing the greatest amount of discomfort. Even during early infancy response to fear or affection is evident. Stroking, patting and tickling result in expressions of satisfaction and contentment. During fear the

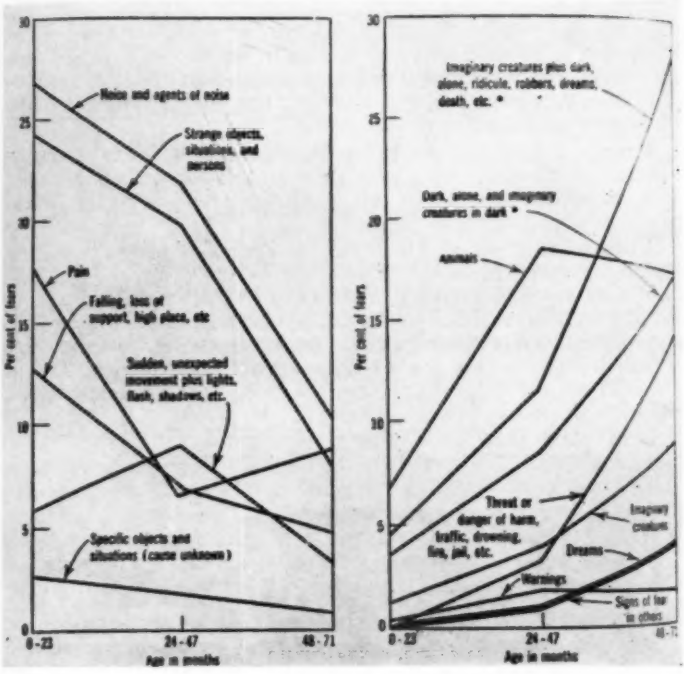

Figure 3

Relative frequency of fears in response to various situations exhibited by children who were observed by parents or teachers. The data include 146 records of observation of children for periods of 21 days (31, 91 and 24 at the respective bi-yearly levels), combined with occasional records of 117 additional children (27, 67 and 23 at their respective levels). From "Children's Fears" by A. T. Jersild and F. B. Holmes, Child Development Monographs, 1935, No. 20. Reproduced by permission of the publisher.

body of a baby may become rigid, the baby may hold his breath, or screaming or kicking may occur. The longer the frustration continues, the more intense the reactions, as a rule.

Ignorant or impatient technologists have frequently used threats as a means of controlling children. It is difficult to conceive anything as improper as using terror as a means of securing obedience in a child. One should never resort to this means of controlling a tot. It is likely to produce two kinds of children —the defiant ones who disbelieve the threats and refuse to be intimidated, and the nervous child who already has innumerable fears.

Many children, who otherwise would be uncooperative, will respond to rewards. For those of normal or above-average intelligence, it is especially intriguing to be allowed to see their own blood counts through the microscope. They find it amusing (and also confusing!) when told that the purple cells are really white ones and the blue cells are red.

A very highly valued prize for little girls is a miniature band-aid with which they may later treat their dollies. No lollipops or sweets should be given unless one is certain of the parent's acquiescence.

Kanner[13] introduced his belief that a child might solve his frustration problem fully if he could derive complete gratification from the aggressive punishment of the frustrator. But this is not practical. Instead, his behavior is censored by adults, leading to greater hostility. In cases of extreme fear, parents could do much to allay the fears by giving the child medical kits as toys. There are kits with nurses' outfits, doctors' instruments, and even microscopes are available at some stores.

Swift[14] says that fear of the strange and unknown, and therefore, dangerous, acquired during childhood, may remain for a time unassociated with any definite object and, hence, produce a state of vague fear. This condition of vague anxiety, however, does not long continue unattached to specific objects. If there is nothing definite to which it can attach itself, the imagination creates something. Fear is the cornerstone of psychopathic diseases. The seriousness of childhood fears lies in their after effects. Children are sensitive to a degree almost incomprehensible to adults.

A childhood experience may leave an indelible mark on his nervous system, and may continue into adulthood. When a needle is pushed into a patient, he is affected not only physically, but psychologically as well. The considerate medical technologist should do everything in his power to alleviate unhealthy fears that may arise in his task of trying to help youngsters.

Acknowledgment: The author wishes to express gratitude to her pathologist, Dr. John D. LeMar, for his kind advice in the preparation of this paper and for the generous contribution of his photography.

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SAINT ALBERT THE GREAT—1193-1280

Selected as Patron of Medical Technologists

St. Albertus Magnus

SAINT ALBERT THE GREAT lived in an age of learned men, the golden age of learning of the 13th Century. In the company of Thomas Aquinas, Bonaventure, Innocent III and others, Albert came to be regarded as the Universal Doctor, because of the extent of his learning. For Albert was not only a renowned philosopher, a celebrated theologian, but also was regarded as above nearly all the men of his time for his knowledge of the natural sciences: physics, biology, mineralogy, chemistry, zoology, physiology and related sciences. He was acquainted with nearly all the known scientific works of the ancients and the accomplishments of his contemporaries. It was Albert who brought into light the works of Aristotle and preserved them for future generations. Thirty-five works of science, of one kind or another, came from the pen of Albert.

Albert's eminent rank among scientists is not based on his discoveries of scientific laws; his distinction is due rather to the fact that he verified by experiment the findings of others in the field and further developed and enlarged them.

—Reprinted from Souvenir Booklet "Patron Saints of Catholic Hospitals."

DEMONSTRATION OF THE "L. E." CELL PHENOMENON*

By KATHERINE DANTZLER, MT (ASCP)
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Acute disseminated lupus erythematosus has been variously described as a generalized vascular disease, a disorder of the collagen system or a "system" disease. The phagocytic "L.E." cell, which is characteristic of the disease, was first described by Hargreaves,[1] who found these cells in heparinized bone marrow preparations from patients with disseminated lupus erythematosus. Hargreaves mentioned in personal letters that upon examination of hundreds of thousands of blood cells he was not able to find L.E. cells in direct smears from untreated peripheral blood, but one case of lupus has been reported in which L.E. cells were found in freshly drawn peripheral blood. This patient was in a terminal stage of the disease and the smears were made only a few days before death. In addition to the L.E. cells reported, other phagocytic cells with nuclear inclusions were found which were not L.E. cells. The author explains that the time factor was likely of prime importance and that the slight trauma of making the smears might have been sufficient to produce the phenomenon.[1]

The L.E. cell of Hargreaves appears as a normal or enlarged polymorphonuclear neutrophil (may rarely be an eosinophile or a basophile) containing a homogenous smoky body without nuclear structure. This inclusion body forces the nucleus of the phagocytic cell to the perimeter, giving a signet-ring appearance. The inclusion body is assumed to be a mature, autolyzed polymorphonuclear neutrophil or lymphocyte. Cytochemical studies[2] by means of Feulgen and methyl green stains indicate that the body contains partly depolymerized desoxyribose and nucleic acid and appears optically and chemically identical with the hematoxylin-staining body described by Klemperer in the study of mesenchymal cells from autopsied cases of disseminated lupus erythematosus.

With some exceptions, most authors agree that the following constituents are needed to produce the L.E. cell phenomenon: (1) the L.E. factor which is present in the gamma globulin fraction of plasma and which is instrumental in the rapid autolysis of leukocytes, (2) phagocytic leukocytes which ingest autolyzed cells, and (3) time outside the body for this process of destruction and ingestion to take place.[3] Most authors agree, also, that there is no satisfactory direct method of making smears for the demonstration of the L.E. cell, but there appears to be no great

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

degree of harmony regarding the necessary amount of time of incubation or whether or not the use of an anti-coagulant is essential. This paper is an attempt to study and summarize several techniques and experiments which are in use. Most of these procedures were executed in this laboratory using blood from a group of eighty "patients," picked at random. Many of these "patients" were completely well—none of them were suffering from lupus erythematosus. These techniques were practiced to determine the time element, ease of performance, effectiveness of cell concentration, laboratory apparatus required, and the general appearance for study of finished preparations. At the end of each procedure a note will appear regarding practical application of that particular method and this author's personal comment.

METHODS

1. *Supravital Staining.*[3] This method was first described by Sabin[4] and uses separate saturated solutions of neutral red and Janus green in neutral anhydrous alcohol (ethyl). Mix 5 parts neutral red to 1 part Janus green in 5 cc alcohol when ready to use. Prepare slides by flooding one side of a clean dry slide with stain. Pass wet side of slide over burner to dry by evaporation. Store slides in dry, rust-proof slide box and use within 1-2 months. When ready to make preparation, prick patients finger and touch clean dry "0" thickness cover slip to drop of blood and drop cover slip onto stained side of slide and rim with vaseline. This method is the same for blood and marrow. Maximum staining and minimal cell damage are observed within 20-40 minutes. Slides may be refrigerated and studied for 8-12 hours.

Note: This method gives nicely stained preparations but the number of leukocytes available for examination is only that which is present in any cover slip smear. However, this method is designed for studying the content of the cell and the author suggests that it is not practical unless employed frequently by observers trained in supravital staining techniques.

2. *Simple Method of Finger Puncture.*[5] Bleed 10 drops of blood from the patient's finger into a clean test tube. Allow the blood to clot for 4-6 hours. Break up the clot with a glass rod and remove mixture of red cells, white cells, and serum to slides and smear. Dry and stain. Examine under low power to locate clumps of leukocytes and examine these clumps under oil immersion. If no L.E. cells are found in these smears, make more smears in the same manner two hours later.

Note: This method is designed to make the test available to the practitioner, internist, and dermatologist without special laboratory equipment. It takes advantage of clotting and incubation

without the inconvenience of venipuncture and centrifugation. However, it was my experience that smears made in this fashion were difficult to examine due to fibrin masses and large clumps of cells and debris. In the thin portions of the smears, leukocytes could be seen clearly and were well-stained, but there was no increase in leukocyte concentration.

3. *Agitation Method.*[6] Using patients with lupus erythematosus and normal controls, 20 cc of venous blood from each patient was put into a clean glass flask containing a bent paper clip and the flask was agitated by hand until the fibrin was collected on the paper clip. At the same time, blood from the same patients and controls was collected in silicone-coated syringes and placed into silicone-coated centrifuge tubes, which were immediately put into the centrifuge and spun at 1000 rpm for 2 minutes. Smears were made from the top of the cell mass in these tubes. The defibrinated blood was also put into centrifuged tubes and spun at 1800 rpm for 5 minutes and smears were made from the buffy coat. The defibrinated blood from the patients with lupus showed many large clusters of neutrophils and many typical L.E. cells. The control bloods showed no clusters and no L.E. cells, but produced well-stained slides with good leukocyte concentration. Serum from the defibrinated blood of the lupus patients was mixed with leukocytes of normal controls, centrifuged immediately, and produced clusters of neutrophils and L.E. cells on smears made from this mixture. Smears from the silicone-treated blood showed clusters and L.E. cells on patients with lupus, though fewer in number than the defibrinated blood, and the silicone-treated controls showed no clusters and no L.E. cells.

Note: This experiment was an effort to prove that an anticoagulant is not necessary to produce the L.E. cell phenomenon, but that it is necessary to concentrate the leukocytes for examination. The authors concluded that mechanical agitation is not essential either, but that it probably helps to increase the number of L.E. cells produced. I found that this technique does produce good leukocyte concentrations and is relatively easy to perform.

4. *A Simple Office Procedure Using Peripheral Blood.*[7] Place 5 cc of blood in a conical centrifuge tube containing 3 drops of liquid heparin (1 cc = 10 mgs). Stopper the tube and shake gently to mix. Allow the tube to stand at room temperature until the cells settle to the bottom. Transfer the supernatant and the top cell layer to another conical tube. Incubate this tube at 37.5° C for 45 minutes or at room temperature for 2 hours. Centrifuge at 1500 rpm for 3 minutes and pipette all

of the buffy coat to slides and make smears. Examine. L.E. cells more often appear at periphery of film.

Note: This experiment was based on the author's belief that L.E. cells may be trapped in the platelet masses. She states that this technique seems applicable to office work because no sternal marrow is needed, no blood or materials from sources other than the patient is needed, heparinized blood can be stored in the refrigerator for as long as 18 hours before the test is completed (if time is scarce), and the results are consistently reproducible. I find that this technique produces good leukocyte concentration and requires a minimum of time and material.

5. *Complement-Fixation Test.*[8] An antigen composed of human leukocytes from normal or granulocytic leukemic blood was used with suspected lupus plasma and the modified Kolmer technique for complement-fixation was employed. This experiment was an attempt to prove an antileukocytic antibody production element of the plasma L.E. factor. It produced positive reactions in 70% of patients with diagnosed lupus erythematosus, but it also was 80% positive in patients who had received multiple blood transfusions. Some positive results were obtained with patients suffering from rheumatoid arthritis, drug hypersensitivity, and chronic agranulocytosis.

Note: We did no experiments in this laboratory using this method.

6. *Simplified Test Using One Drop of Finger Blood.*[9] Prepare slides by placing drops of normal polymorphonuclear leukocyte concentrations upon clean slides and allow to dry. Prick the patient's finger and place one drop of blood upon the accumulation of dried cells. Use an applicator to mix the fresh blood with the dried cells, place another clean slide on top of the mixture, and pull apart to make two smears. Fix and stain. Examine for L.E. cells. There may be a slight difference in the appearance of the inclusion body in the L.E. cells found with this method, but they are easily recognizable.

Note: This method is based on the author's theory that in order to produce the L.E. cell phenomenon, it is necessary to bring together living polynuclear leukocytes, dead cells, and lupus serum. When this is accomplished, the lupus serum can depolymerize the desoxyribose nucleic acid of the nuclei of the dead cells but not the living ones. The depolymerized material is then phagocytized by the living polynuclear cells and formation of L.E. cells results. This technique eliminates venipuncture, centrifugation, incubation, and hours of searching for the cells. I found that after a great deal of practice I was able to make smears thin enough to be stained and examined. We report no

false positives in control patients and consistently reproducible results.

Recent experiments[10] show evidence to suggest an identity of the L.E. cell phenomenon with desoxyribonuclease, an inhibitor found in mammalian tissues. This technique consists of incubating together the serum from a patient suspected to have lupus and "sensitized" leukocytes. These sensitized cells are prepared by washing leukocytes four times in isotonic saline. The sensitized cells and the patients serum are mixed in equal portions and incubated at 37° C for 1 hour. The mixture is then centrifuged at low speed for 5 minutes and smears are made from the cell layer, fixed in methyl alcohol, and stained in dilute Giemsa stain for thirty minutes. This technique is based on the theory that the L.E. plasma factor which contains no DNase activity, causes the release of the intracellular DNase, making the L.E. cell phenomenon possible by removing the inhibitor, thus producing L.E. cells from the normal leukocytes employed in the test.

Dubois[11] emphasizes the study for the L.E. cell phenomenon on all patients with evidence of any form of increased splenic activity, especially leukopenia, thrombocytopenia, and hemolytic anemia, and Weiss and Swift[12] stress the importance of the search for L.E. cells on patients with rheumatoid arthritis if they present suggestive symptoms. These authors found L.E. cells only when lupus was the leading or possible diagnosis, but in a study of 350 patients they found some other changes in these bloods, such as the "tart" cell. Some L.E. cells were reported in three of sixteen bloods of patients receiving hydralazine but no L.E. cells were found in patients with penicillin hypersensitivity. However, since lupus erythematosus is regarded as possibly a hypersensitivity phenomenon, L.E. cells might be found in severe and long-standing penicillin reactions. The authors feel that L.E. cells reported in fungus diseases and in some other rare cases are really so-called "tart" cells, histocytes which have engulfed a dead or damaged white cell which is usually a lymphocyte. This ingested body retains some of its nuclear outline and chromatin pattern, while the true L.E. cell has a homogeneous smoky body, without nuclear structure. Tart cells may be percursors or obortive forms of L.E. cells, but they must not be regarded as evidence of lupus unless typical L.E. cells are also found.

These are just a few of the available methods for demonstrating the L.E. cell phenomenon. There are many more, but the responsibility of the medical technologist is to use an accepted effective technique, produce a good preparation, and be prepared to devote the goodly amount of time which is required to examine it.

SUMMARY

The presence of clumping rosettes and the typical L.E. cell found in blood and marrow preparations may be taken as conclusive evidence of systemic lupus erythematosus.[12] The L.E. cell phenomenon is almost always positive at some stage of the disease and is of particular importance in borderline cases. This paper is a review of some research experiments and of several accepted methods for demonstrating the L.E. cell phenomenon.

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TREPONEMA PALLIDUM IMMOBILIZATION TEST*

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It is well established that animals and human beings infected with Treponema pallidum become resistant to reinfection with this organism, but the mechanism of this immunity is poorly understood.[1] Studies on this problem have been seriously handicapped by the lack of in vitro methods for the detection of antibody to Treponema pallidum because this organism has not as yet been cultivated and is therefore not available in adequate amounts for the usual in vitro immunological studies. However, a method has been developed which keeps these anaerobic organisms alive and virulent for several days. I will discuss this in more detail a little later.

While standard serological tests for syphilis, carried out with lipoidal antigens from beef heart or other mammalian tissues, serve to detect the presence of syphilitic infection with a relatively high degree of specificity, they do not provide an index of immunity to the disease.[1] Proof of this is found in the decline in the titre of Wassermann antibody or "reagin," with progression of syphilis into the latent stage, although it is during this stage that animals exhibit a high resistance to reinfection.

Techniques have been developed in which virulent Treponema pallida are extracted from rabbit testicular syphilomas in a relatively tissue-free state and maintained in vitro in a highly active state for several days. With these live organisms, it has become possible to demonstrate the presence of an antibody in sera from syphilitic animals and human beings which immobilizes virulent Treponema pallida in vitro. Nelson discovered this immobilizing antibody in 1947.[4]

There are two types of antibody in syphilis:

1. Lipid type—gives the reaction in Serologic Tests for Syphilis and is called Reagin.

2. Immobilizing antibody—gives the reaction in T.P.I. It is this latter antibody that we are here concerned with, and in July, 1948, Nelson made the first antibody run, which we now know as the Treponema Pallidum Immobilization Test.[3]

MATERIALS AND TECHNIQUES FOR THE TEST[1]

Preparation of Treponeme Suspensions:

Rabbits to be used as source animals are inoculated intratesticularly with 0.5 ml of a suspension of Treponema pallida freshly isolated from rabbit testicular syphilomas. After 7 to 14 days, the testes are removed and immediately cut in thin slices

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956. Scientific Products Foundation—3rd Award Serology.

and then washed with chilled 0.85% saline to remove loose tissue particles. The slices are placed in basal medium under an atmosphere of 5% carbon dioxide and 95% nitrogen, and the treponemes extracted from the tissue with gentle rocking for approximately 2 hours at 35° C. The extracted treponemes are separated from tissue debris and spermatozoa by filtration through a Corning "medium" fritted disc. The filtrate so obtained is slightly opalescent and on darkfield examination shows two or more organisms per field and an occasional red blood cell, but no appreciable tissue debris. As determined by calibration of the microscope, two treponemes per field is equivalent to 10,000,000 organisms per ml. The percentage of motile organisms is also determined at this time by examining fifty successive treponemes in fields selected at random, and this is recorded as the O-hour reading. Ordinarily, 90 to 98% of the organisms exhibit active motility.

Source of Complement:

The source of complement is pooled guinea pig serum, collected and distributed under aseptic conditions in cotton-plugged, rubber-capped tubes which are stored in solid carbon dioxide.

Collection of Sera:

Tests for immobilizing activity have been performed on five groups of sera: 1) from non syphilitic, apparently healthy rabbits; 2) from untreated rabbits infested with Treponema pallidum for 3 to 9 months; 3) from persons whose histories were reliable with regard to the absence of syphilitic infestion; 4) from patients with darkfield positive primary or secondary syphilis; and 5) from presumably non-syphilitic patients, either with acute febrile diseases or with chronic allergic diseases. Serum, obtained aseptically, is stored at —20° C in cotton-plugged, rubber-capped tubes. Samples to be tested are heated at 56° C for 30 minutes just prior to use.

Test Procedure:

A simplified formula for the test is as follows: T. pallidum emulsion in artificial medium + complement + known syphilitic serum $\xrightarrow[15\text{-}18 \text{ hours}]{\text{incubated at } 35^\circ \text{ C}}$ treponemes killed or immobilized. In more detail, 1.7 ml of the filtered treponeme suspension, 0.2 ml of the serum to be tested, and 0.1 ml of complement (guinea pig serum), are mixed. In each experimental series a control tube containing 1.7 ml of treponeme suspension, 0.1 ml of complement, and 0.2 ml of ultrafiltrate of serum is included. The tubes containing these mixtures are incubated at 35° C in a Brewer anaerobic jar filled with a gas mixture of 5% carbon dioxide and

95% nitrogen. Percentages of motile organisms are determined at intervals of 4 or 8 hours, depending on the nature of the individual experiment, for a total period of 16 hours.

At the end of each experiment all mixtures of treponemes, serum, and complement which fail to produce immobilization should be tested for the presence of active complement by the addition of sheep red blood cells sensitized with hemolysin. This step is necessary in order to rule out false negative results due to anticomplementary effect.[1] If active complement is present, the red blood cells would be hemolyzed.

"Since all the organisms seen are either fully active or completely non-motile, their differentiation presents no difficulty."[1] Treponemes immobilized by antibody and complement are not appreciably distorted and they may exhibit any of the several shapes described for motile Treponema pallidum. Unlike treponemes observed in material from human or rabbit syphilitic lesions, however, those suspended in the fluid medium show extremely active movements, and translation (to-and-fro movements) is infrequent. Most of the organisms contract and relax rhythmically in a relatively confined area. This lack of translation is beneficial in that it guards against repeated counting of any one motile treponeme in adjoining fields and makes it possible to make accurate determinations of the total number of organisms, as well as of the percentage of motile organisms.

Comparison of the Action of Normal and Syphilitic Rabbit Serum on Treponema Pallidum in Vitro, With and Without Complement. Motility of Treponemes at O-Hour: 98%[1].

Serum Pool Tested	Final Serum Dilution	Motility of Organism After Incubation at 30° C.			
		Without Complement		With Complement 1/20	
		12 Hrs.	24 Hrs.	12 Hrs.	24 Hrs.
		%	%	%	%
Normal	1/10	92	94	96	84
Normal	1/100	92	94	96	92
Normal	1/1000	90	94	96	92
Syphilitic	1/10	96	84	2	2
Syphilitic	1/100	92	96	50	10
Syphilitic	1/1000	96	92	92	84
Serum Ultrafiltrate Control	.....	94	96	92	92

With reference to the table, neither serum pool exerted a significant immobilization of Treponema pallidum in the absence of guinea pig complement. However, when complement was present, a marked reduction in motility of the organisms was produced by the syphilis serum pool, but not by the normal serum pool. "Since this effect was manifested only in the presence of

complement, it is highly probable that the component of syphilis serum responsible for the immobilization is an antibody against Treponema pallidum."[1]

The Rate of Immobilization as a Function of Time and Serum Concentration:

In the presence of complement, the immobilizing effect increased with time and with serum concentration. With occasional normal sera, a small degree of immobilization occurred at a final concentration of 20%, but none of the normal sera produced significant immobilization at a concentration of 10%. Therefore, a final serum dilution of 1:10 and a minimal time of 16 hours' incubation have been used in these experiments.[1]

The Rate of Immobilization as a Function of Temperature:

The rate of antibody action increased with temperature, and as a result the sensitivity of antibody detection at any given time interval also became greater. However, significant immobilization of the control suspension was manifest as early as 4 hours at 40° C, but not at 35° C or lower. Therefore, an incubation temperature of 35° C was chosen for these experiments.[1]

The Role of Complement and the Influence of its Concentration on the Rate of Immobilization:

Heat inactivation or removal of complement by absorption with a specific precipitate renders guinea pig serum incapable of immobilizing Treponema pallidum in conjunction with syphilitic rabbit serum. In view of these findings, the immobilizing activity of fresh guinea pig serum has been attributed to its complement content.[1]

Immobilization as a Criterion for Treponemicidal Activity:

In previous experimentation carried out on the survival of Treponema pallidum in vitro, data were accumulated which demonstrated that motile organisms retain their virulence. In this study it was necessary to establish the converse—that nonmotile organisms are dead, in order to prove that the effect of the antibody is treponemicidal. Experiments indicated that Treponema pallidum immobilized by antibody and complement are non-infectious and presumably dead.[1]

The Relationship of Immobilizing Antibody to Reagin:

In order to study the relationship of reagin to immobilizing activity of syphilis serum, absorption experiments were carried out. Absorption of the syphilis serum with Eagle antigen reduced the reagin titre to nothing. However, no detectable decrease in the immobilizing activity resulted. The T.P.I. titre remained high, and therefore it may be concluded that the

immobilizing and reagin activities are due to separate antibodies.[1]

Of interest is the fact that the reagin titre reaches its peak the third month following infection, then starts a gradual decline, leveling off at a low titre after six months, and eventually drops to zero. The T.P.I. antibody titre, however, reaches its peak about the tenth month and then remains at a high titre, and it is thought that this high titre persists for life.

The Relative Thermostability of Reagin and Immobilizing Antibody:

There is a progressive drop in reagin titre with an increase in temperature, but no corresponding decrease in immobilization activity with increase in temperature.[1] This is further proof that these are separate antibodies.

Some Test Results:

1. Twenty cases of secondary syphilis all showed a marked degree of immobilizing activity, and only 10 out of 20 showing primary syphilis exhibited immobilization.[2] This is probably due to the fact that the antibody titre increases with the duration of the disease.

2. Survey of Sera from Healthy Persons:

Twenty normal sera showed no immobilization in one study, and eighty out of eighty-one normal sera showed no immobilization in another study.[2]

3. Survey of Sera from Patients with Diseases Other than Syphilis:

In one study of 16, all were negative. In another study, 87 patients were also found to be negative. Of the several diseases studied in relation to the immobilization of Treponema pallidum by the patient's serum, three gave unsatisfactory results. These were one patient with chicken pox, one patient with atypical pneumonia, and one with infectious mononucleosis.[2]

4. Survey of Sera from Patients with Probable Latent Syphilis:

Fifty negro patients having latent syphilis all showed a strongly positive immobilization test.[2]

The T.P.I. test not only shows high specificity for T. pallidum infections, but also for those caused by the other members of the genus Treponema. Sera from patients infected with T. pertenue (yaws), and T. carateum (pinta) have also been tested and found to give a positive reaction with the T.P.I. tests.[4] This would suggest the possibility that a common antibody exists within the genus.[2]

Unfortunately, the immobilization test is expensive and time-consuming, and requires the use of sterile serums and cerebrospinal fluids. Also, it is complicated and subject to numerous technical errors, and it is not yet ready for general adoption. Therefore, it is of little practical value for checking biologic false positive and doubtful serologic reactions.

Of particular interest to me, however, is the fact that there are two antibodies, as it were, produced by syphilis and that further study with the T.P.I. test may some day bring about a method for ruling out the bugaboo of serologic tests—the false positive.

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THE DETECTION OF BLOOD IN URINE*

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INTRODUCTION

Many times there is no indication as to whether the term hematuria as it appears in case histories refers to blood which can be recognized by gross inspection of the urine or refers to blood recognized in microscopic examination of urine sediment. Furthermore, the relative sensitivity of gross and microscopic tests is not defined in most discussions of the subject. Chemical tests for blood in urine have never been widely used in clinical practice, although most texts of clinical pathology or medical biochemistry mention such tests. In general, chemical tests for blood have the reputation of being extremely sensitive, but when such tests are applied to urine they have been regarded as having a minimal sensitivity and utility.[1] Recently, an improved chemical test, called Occultest,‡ has been introduced for the detection of occult blood in urine. The present report describes comparative studies of the detection of gross hematuria, microscopic hematuria and blood detection in urine with several chemical tests including Occultest.

METHODS

Normal Urines. To establish standards of comparison, blood was first added to urine specimens from ten normal healthy subjects. Each specimen had a volume of approximately 500 ml. The pH of these urines ranged from 5 to 6.5 and the specific gravity from 1.009 to 1.025.

Abnormal Urines. There were eight different types of abnormal urines in this series. In each case urines from two different subjects were used, thus giving a total of sixteen different specimens. These abnormal urines were obtained as follows:

1. *Low specific gravity.* Two subjects drank a large amount of water. Resulting urine specimens had specific gravities of 1.002 and 1.003.
2. *Ascorbic acid—high normal.* Each subject took one multiple vitamin tablet (containing 40 mg. of ascorbic acid) three times in one day. The urines had ascorbic acid levels of 137 and 74 mg. per liter.
3. *Ascorbic acid—therapeutically high.* Each subject took a 500 mg. portion of ascorbic acid with each meal and before retiring. Urines were obtained with ascorbic acid levels of 1360 and 1070 mg. per liter.

* Read before First North American Conference of Medical Laboratory Technologist, Quebec, Canada, June, 1956.
‡ Occultest is a trademark of Ames Company, Inc., Elkhart, Indiana.

4. *Acid urine.* One subject ingested 5 gms. of ammonium chloride and the other subject 7.5 gms. Urines had pH values of 4.85 and 4.80.
5. *Alkaline urine.* One subject took 10 gms. of sodium citrate and the other 15 gms. Urines had pH values of 8.3 and 8.5.
6. *Alkaline urine after bacterial decomposition.* Two normal urines were inoculated with a small amount of badly decomposed alkaline urine from a hospital patient. After incubation both urines had pH values of 9 and heavy growth of bacteria.
7. *Urea added to low specific gravity urines.* Urea was added to each urine to give a concentration of 5% urea. This changed the specific gravities from 1.001 to 1.014 and 1.003 to 1.016.
8. *Urea added to normal urine.* Five per cent urea was again added to two urines. In these cases the specific gravities were changed from 1.016 to 1.029 and from 1.014 to 1.027.

Addition of Blood to Urine. Each one of the twenty-six urines (10 normal and 16 abnormal specimens) was treated in the same general way. A red blood cell count and a hemoglobin value were obtained on freshly drawn blood. The blood was then added to a portion of urine to give a dilution of one part of blood in 125 parts of urine. Serial dilutions were made to give the series of twelve dilutions shown in Figure I.

Detection of Gross Blood. Thirty-milliliter portions of each urine specimen were shown in 225 ml. heavy glass hospital-type specimen bottles about six inches tall. The bottles were coded and mixed. One bottle at a time was shown to five different observers. In some cases a sample was shown more than once to the same observer. Each person was asked whether he could see blood in the sample. After all urine samples in the series had been viewed individually, they were shown next in pairs to the same five observers. Each pair of urines consisted of a sample with blood and the one without blood.

Microscopic Detection of Blood. Microscopic examination of urine sediment for red blood cells was made on all dilutions from 1:30,000 through 1:1,000,000 and on the urine without added blood. The following procedure was used:

> Place 15 ml. of well-mixed urine in a conical centrifuge tube marked at 15 ml. and at 0.25 ml. and centrifuge for 5 minutes at 2000 RPM. Immediately remove the supernatant by suction to the 0.25 ml. mark. Suspend the sediment in the remaining 0.25 ml. by thorough mixing and, using a micropipette, place 0.020 ml. of sediment suspension on a glass slide and cover with a 22 x 22 mm. cover slip. Count the number of red blood cells in 10 fields under the high dry power objective. The microscope used in this study gives a

magnification of 430x under this objective. The average number of RBC/hpf is determined by dividing the total number of red blood cells counted by 10 (the number of fields counted). In this study each of two operators made and counted one slide from the same tube of sediment.

Using this precise method, the number of red blood cells in 1 ml. of any given urine specimen can be calculated by multiplying the average RBC/hpf by 4000. The method of arriving at this factor of 4000 is as follows: (Calculations are valid only for this method of microscopic detection and for a microscope which gives a magnification of 430x under the high dry objective.)

Diameter of high power field = 0.35 mm. (measured with Nebauer-ruled counting chamber.) Area of high power field = 0.096 sq. mm. Area covered by cover slip (22 x 22 mm.) = 484 sq. mm. 484/0.096 = 5040 high power fields under cover slip. Sediment volume under cover slip = 0.020 ml. which corresponds to 60 times as much original urine or 1.2 ml. (since 15 ml. urine yields 0.25 ml. sediment or a 60-fold concentration of urine) 1.2 ml. urine for 5040 high power fields = 1 ml. for approximately 4000 fields.

Chemical Tests for Blood. Six different types of chemical tests were run by each of two operators. Each operator recorded the highest dilution of blood in urine which gave a positive reaction with each of the six different chemical tests. An attempt was made to bracket the dividing line of sensitivity with two dilutions giving positive reactions and with two dilutions giving negative reactions. The method used for each of the chemical tests is described below:

1. *Benzidine dihydrochloride test.* Place 1 ml. of urine in a test tube and dilute to 5 ml. with water. Add in the order named, 1 ml. of 1% aqueous benzidine dihydrochloride solution, 1 ml. of 3% hydrogen peroxide solution, and 1 ml. of 1% sodium acetate solution. A blue or green color develops in the presence of blood. (These directions accompany Merck Reagent Benzidine Dihydrochloride.)
2. *Guaiac tests.*[2] Place 1 ml. of urine in a test tube. Add 0.5 ml. of glacial acetic acid. Add 1.5 ml. of freshly prepared gum guaiac reagent. A blue color develops in the presence of blood. The reagent is prepared by dissolving 200 mg. of gum guaiac in 12 ml. of 95% ethyl alcohol and then adding to it 24 ml. of 3% hydrogen peroxide.
3. *Benzidine base test.*[3] To 1 ml. of glacial acetic acid, add a small amount of benzidine base (about size of pea) and 1 ml. of 3% hydrogen peroxide. To the mixture add 3 drops of urine. The formation of a green or blue color indicates the presence of occult blood.
4. *Orthotolidin test.*[4] To 1 ml. of urine in a test tube, add 1 ml.

of 4% orthotolidin base dissolved in glacial acetic acid and 1 ml. of 3% hydrogen peroxide. The presence of blood is indicated by a blue or green color.

5. *Occultest.* This is a specific chemical test designed for the detection of occult blood in urine. Place one drop of urine on one of the squares of filter paper supplied by the manufacturer. Place a reagent tablet on the moistened area and add two drops of water. The appearance of a blue color on the paper within two minutes indicates the presence of blood. If no color is seen on the paper at the end of two minutes, the test is negative.
6. *Zwarenstein's test.*[5] Place a knife-point of solid orthotolidin dihydrochloride in a small test tube. Add 5 drops of urine and mix well. Place one drop of this mixture on a small piece of Whatman No. 1 filter paper on a clean porcelain tile. Add one drop of Zwarenstein's reagent (a solution containing equal parts of 3% hydrogen peroxide and glacial acetic acid allowed to stand at least 24 hours before using). An immediate blue-speckled coloration of the paper indicates the presence of blood.

RESULTS

Gross Blood

Series (1)—Observations on paired samples. The dictionary defines gross and macroscopic as large enough to be seen by the naked eye. If blood is added to normal urine and the eye can detect a difference between the two specimens, then in the strictest sense of the word this can be called gross blood. Each observer was shown two specimen bottles at a time and asked if he could observe a difference and if so, which urine contained added blood. Under these conditions blood was detected with 100 per cent accuracy when one part of blood was contained in 2000 parts of urine. The 1:4000 dilutions were differentiated 86 per cent of the time and the 1:8000 dilutions 72 per cent of the time. At a dilution of 1:16,000 less than half of the observers could tell a difference between the negative and positive urine when held side by side. In two per cent of the observations the negative urine was called positive and the 1:16,000 dilution negative. Line 1 in Figure I shows results obtained for all dilutions. The prevalence of wrong guesses (negative urine called positive) is indicated by the shaded area.

Gross blood detection in the 16 abnormal urines showed no difference from the detection of gross blood in the normal urines. All values fell within the ranges noted for the normal urine series.

Series (2)—Observations on single samples. Before viewing the previous series as pairs, each specimen was first shown individually to the five observers. The sensitivity for gross blood

detection was less when there was no negative urine for side by side comparison. With samples viewed singly, only the 1:125 dilution was detected 100 per cent of the time. The 1:2000 dilution was reported positive 62 per cent of the time in this series as compared with 100 per cent in the first series. There seemed to be less straining to find positives since in this series no negative urine was ever called positive. All values for this series are plotted as line 2 in Figure I.

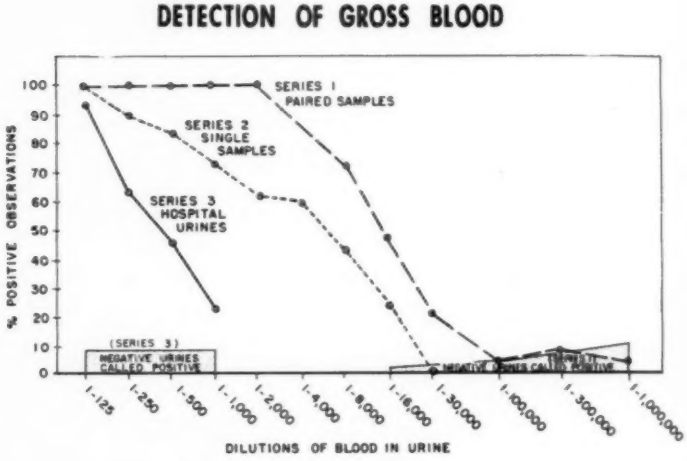

Figure I

In the detection of gross blood, the highest degree of sensitivity is obtained when the unknown sample is compared with a negative control urine (Series 1). The sensitivity is greatly reduced when the unknown is viewed without a negative urine for comparison (Series 2). A very low degree of sensitivity results when unknowns are included in a series of abnormal urines from hospital patients (Series 3).

Results for gross blood detection in the sixteen abnormal urines all fell within the ranges found for the detection of gross blood in normal urines.

The normal series of urines with samples mixed and viewed singly still does not give a practical indication of the amount of blood that can be detected by the naked eye. The urines were far from being perfect blind samples. The observers were influenced by two factors. In the first place they knew that all urines were from normal healthy subjects. Secondly, they knew that the one variable in the series was added blood. Any deviation from the normal appearance of the urine could be explained only by the presence of blood. Therefore, the observers, whether

they were aware of it or not, were actually biased in their judgment.

Series (3)—Observations on hospital urines. The abnormal appearance of a urine specimen as seen in a clinical laboratory cannot be attributed so simply to the presence of blood. Urine may be brown, red, or turbid for completely different reasons. A total of 247 observations were made on negative hospital urines (some of which looked suspiciously as though they contained blood) and 315 observations on urines with added blood. Under these conditions the 1:125 dilution was detected 94 per cent of the time, the 1:250 dilution 63 per cent of the time, the 1:500 dilution 46 per cent of the time, and the 1:1000 dilution only 23 per cent of the time. Furthermore, 9 per cent of the negative urines were called positive. Data from this experiment are shown as line 3 in Figure I. The negative urines called positive are indicated by a shaded area.

Microscopic Examination of Urine Sediment

Normal urines with blood added. Microscopic examination of urine sediment for red blood cells was carried out as described in the section on methods. Since red blood cell counts were carried out on each blood which was added to urine, it was possible to calculate the amount of blood recovered by microscopic examination. The average values for each dilution for the series of normal urines are shown in Table I. Recoveries ranged from 32 per cent to 74 per cent at a dilution of blood in urine of 1:30,000; from 30 per cent to 83 per cent at a dilution of blood of 1:100,000; from 35 per cent to 85 per cent at a dilution of 1:300,000; and from 18 per cent to 130 per cent at a dilution of 1:1,000,000. The *average* recovery at a dilution of 1:1,000,000 is close to the *average*

TABLE I
Microscopic Recovery in Urine Sediment of Red Blood Cells Added to Normal Urine

Blood Dilution	Average RBC/ml. From Added Blood	Average RBC/hpf Microscopic	Average RBC/ml. Microscopic	Average Per Cent of Recovery
1:30,000	219,000	28	112,000	51
1:100,000	66,000	9	36,000	54
1:300,000	22,000	3	12,000	55
1:1,000,000	6,600	1	4,000	61
None	0	0.3	.....	..

recovery at each of the other dilutions though the range is much wider. However, it is felt that the microscopic examination of urine sediment cannot accurately detect the difference between a single normal urine and the same urine with blood added at a concentration of 1:1,000,000, even though this method of carrying out microscopic examination of urine sediment is more precise than that usually used in clinical or hospital laboratories. Precise measurements were used in this procedure only to obtain

data which would be valuable in determining the sensitivity of microscopic detection of occult blood. It is felt that microscopic examination of urine sediment will easily detect one part of blood in 100,000 parts of urine, and from this small series will sometimes detect one part of blood in 300,000 parts of urine. However, it must be emphasized that variations in urine, particularly specific gravity and pH, will greatly affect the sensitivity of the test as will be discussed further.

Abnormal urines with blood added. In studying the series of abnormal urines, it was found that the urinary factors which significantly decrease the number of red blood cells found in urine sediment after addition of blood were low specific gravity

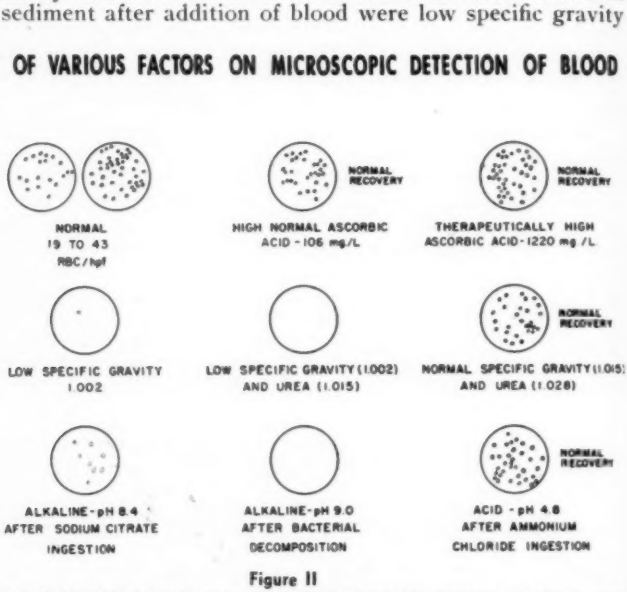

Figure II

Microscopic fields observed with urine sediment obtained by standard technique after the addition of blood to various urines in a dilution of 1:30,000.

and high pH. The effect of the various factors included in this study is shown pictorially in Figure II. These pictures represent the number of red blood cells per high power field obtained in urine sediment after addition of blood to urine at a dilution of 1:30,000. Comparison of further dilutions showed the same effect, though naturally not so marked because of the fewer number of cells found in normal fields. It is apparent that urine with specific gravity of 1.002 hemolyzed added blood. Quantitative

chloride values on the low specific gravity urines ranged from 0.06 per cent to 0.2 per cent NaCl so that hemolysis of blood by low specific gravity urines is due to the hypotonicity of such urine. It will also be seen that the addition of urea to bring the specific gravity of such a urine to 1.015 (about 5 gms. urea per 100 ml.) did not prevent hemolysis of blood. On the other hand, the addition of the same amount of urea to a urine of normal specific gravity did not cause hemolysis. Therefore, it may be assumed that the concentration of urea in urine has little effect one way or the other on the extent of hemolysis of added blood.

Urines with a pH of 8.4 obtained after ingestion of sodium citrate partially hemolyzed added blood. The cells which were still visible were faint and enlarged ghost cells. Normal urines which stood at room temperature for 3 days to allow bacterial decomposition to take place also hemolyzed added blood because of the increase in alkalinity (pH rose from 6 to 9).

It will also be seen from Figure II that a high normal or therapeutically high ascorbic acid concentration in urine or a low pH after ingestion of ammonium chloride did not significantly change the amount of blood recovered from urine sediment.

In two samples of our normal series, it was noted that the red blood cells hemolyzed on the slide during the actual counting procedure. In such cases, specific gravity was normal (1.025, 1.014) and pH was normal (6.5, 6.0). The sodium chloride content of the first urine was 0.9% so that there was no apparent single cause for hemolysis. The second urine contained only 0.4% sodium chloride. This may have been low enough to cause hemolysis, though it is interesting to note that hemolysis did not occur until the sediment was placed on the slide. (Another urine of the normal series also contained 0.4% sodium chloride and did not show this phenomenon.) The sediment from the centrifuged urines looked normal in that there were packed red blood cells in the centrifuge tubes. For a few fields, cells were visible on the slide, then they began to fade into ghost cells and finally disappeared altogether. Replicate slides were made using clean pipettes, slides and cover slips and the same results were obtained. Counts on these urines were obtained by counting a few fields per slide until 10 fields had been counted.

It is apparent that there are various factors which decrease the number of added red blood cells recovered by microscopic examination of urine sediment. Comparison of the results of microscopic counts and of Occultest shows that though both have about the same sensitivity, the chemical test gives more uniform results since there are no known interfering factors.

Chemical Tests for Blood

Normal urine with blood added. The sensitivity of the six chemical tests for blood varied quite widely. For example, a 1:1000 dilu-

tion of blood in urine was usually the highest dilution to give a positive reaction with the benzidine dihydrochloride test. It was found that Zwarenstein's test on the other hand gave trace reactions with 25 per cent of the urines from normal healthy subjects. The next most sensitive test was Occultest. In most cases a trace reaction was obtained with Occultest when one part of blood was present in 300,000 parts of urine. Occultest was about 300 times as sensitive as benzidine dihydrochloride. The sensitivity of Occultest approximated the sensitivity of the microscopic examination. In the middle area of sensitivity were guaiac, benzidine base, and liquid orthotolidin tests. The relative sensitivity of the six chemical tests is shown in Figure III. In this figure the sensitivity is expressed as the largest quantity of urine (average value for 10 normal urines) which will give a positive reaction after the addition of one drop of blood. In Figure IV

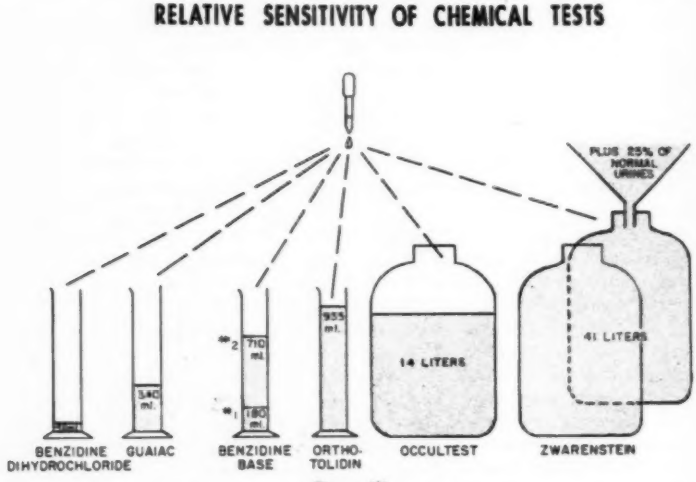

Figure III

The largest quantity of normal urine which will just barely give a positive reaction after the addition of one drop of blood indicates the sensitivity of each method.

the sensitivity of each test is shown, and the range for each test serves as a reference for abnormal urines.

The wide range of sensitivity found with the different chemical tests cannot be explained simply by the kind of chemical reagent used. Occultest contains orthotolidin base, yet it is 15 times as sensitive as the liquid orthotolidin test which was included in this series. Operator 2 reported that the benzidine base test was 4 times more sensitive than Operator 1 reported. This difference

was very simply explained by the fact that Operator 1 thought peas were smaller. Less benzidine base was used and the result was a less sensitive test. Either operator could agree with the other operator merely by shifting the amount of benzidine base. In Zwarenstein's test, orthotolidin dihydrochloride is used. This does not indicate that this particular chemical is inherently the most sensitive for the detection of blood in urine. Nor is benzidine dihydrochloride inherently the least sensitive. If benzidine dihydrochloride is substituted for the orthotolidin dihydrochloride in Zwarenstein's test, a very sensitive test can be obtained.

The sensitivity of any chemical test is mainly a reflection of the technique or method used, the way the various chemical components are combined, and the amount of chemical used. Apparently, tests run on paper are much more sensitive than tests run in test tubes. Contrary to current beliefs, the actual chemical used appears to be of minor importance in determining the ultimate sensitivity of any given test.

Abnormal urines with blood added. The chemical tests where color was developed on paper were apparently unaffected by the types of abnormal urines tested. The sensitivity of Occultest and Zwarenstein's test remained within normal limits with all abnormal urines. Chemical tests carried out in test tubes, however, were dramatically affected by some of the abnormal urines.

The benzidine dihydrochloride test, a relatively insensitive test initially, became even less sensitive when used to test urines with therapeutically high levels of ascorbic acid or highly alkaline urines such as result from bacterial decomposition. There was a suggestion of increased sensitivity with low specific gravity urines with or without added urea.

The guaiac test in the presence of bacterial decomposition gave false positive reactions with urine even before addition of blood. The sensitivity of the guaiac test was somewhat decreased by high normal levels of ascorbic acid and radically decreased by therapeutically high levels of ascorbic acid. There was also a suggestion of decreased sensitivity with acid urines obtained after ammonium chloride ingestion.

With the benzidine base test, both operators found that the sensitivity was somewhat decreased in the presence of high normal ascorbic acid concentrations and radically decreased in the presence of therapeutically high ascorbic acid levels. Operator 1 who used smaller amounts of reagent found a tendency toward increased sensitivity when testing low specific gravity urines with or without added urea. Operator 2 found a tendency toward decreased sensitivity in acid urines after ammonium chloride ingestion.

The liquid orthotolidin test was also affected by ascorbic acid. The sensitivity of the test was somewhat decreased by high

normal levels and radically decreased by therapeutically high levels. There was a suggestion of increased sensitivity when testing low specific gravity urines.

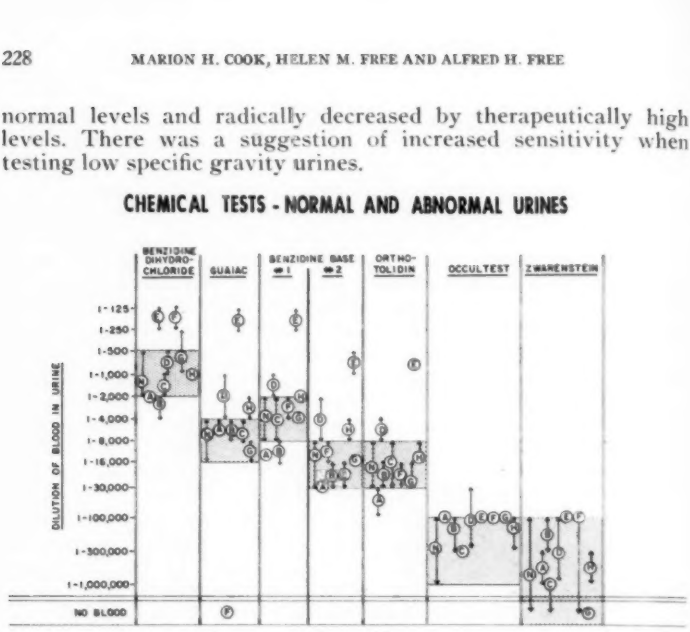

Figure IV

The highest dilution of blood in normal and abnormal urine which will just barely give a positive reaction is shown for each type of chemical test. The position of the circle indicates the average sensitivity value while arrows show the range of sensitivity. The shaded area is the range for normal urine with blood added. The code letter within each circle indicates the type of urine to which blood was added. N = normal, A = low specific gravity, B = low specific gravity with added urea, C = normal specific gravity with added urea, D = high normal ascorbic acid, E = therapeutically high ascorbic acid, F = alkaline after bacterial decomposition, G = alkaline after sodium citrate ingestion, and H = acid after amonium chloride ingestion.

The effect which each type of abnormal urine has on the sensitivity of each of the various chemical tests is given in Table II, and shown graphically in Figure IV.

DISCUSSION

The word "hematuria" may be used to describe a urine sample which contains more blood than urine. In contrast, the term may also be applied to a urine sample in which only one part of blood is present in 300,000 parts of urine. With such extremes it is natural that the means of recognizing hematuria may vary. It is important to know something about the interrelationships of gross hematuria, microscopic hematuria and positive chemical tests for occult blood in the urine. These interrelationships are shown in Figure V. It is apparent that most of the chemical tests fall in a range where the microscopic examination of sediment might reveal "innumerable" cells per high power field. Only Occultest is

sufficiently sensitive to give a positive reaction in a range where only a few red cells might be present in a high power field.

Visual recognition of gross hematuria is readily and accurately accomplished when there is one part of blood in 125 parts of urine. As amounts of blood decrease from this value the certainty of recognition diminishes with the procedure of examination playing an important role in the sensitivity and accuracy achieved. A comparison of values shown in Figure I readily explains how conflicting opinions may develop regarding the sensitivity and accuracy of visual recognition of gross hematuria.

Microscopic examination of urine sediment is not a standardized technic from one laboratory to another. Even the magnification achieved per high power field varies quite markedly with different microscopes. In the present study, using a standardized technic for microscopic examination, it was shown that variations in urine which frequently are encountered may alter results. Low specific gravity (or more exactly, low salt concentration) or high pH may cause hemolysis of blood cells in urine so that they

TABLE II

Changes in Sensitivity by Various Urinary Factors on Various Methods for Detection of Blood in Urine (Decreased = Decreased Sensitivity; Increased = Increased Sensitivity; O = No Effect; Decreased? = Suggested Decreased Sensitivity; Increased? = Suggested Increased Sensitivity; ? = Effect Undeterminable.)

Urine Factors	CHEMICAL DETECTION							
	Macroscopic or Gross Detection	Microscopic Detection	Benzidine Dihydrochloride	Guaiac	Benzidine Base	Orthotolidin Base	Occultest	Zwarenstein*
Low Specific Gravity	O	Decreased	O	O	Increased?	Increased?	O	O
Low Specific Gravity + Urea	O	Decreased	Increased?	O	Increased?	O	O	Decreased?
Normal Specific Gravity + Urea	O	O	O	O	O	O	O	*
Alkaline (Sodium Citrate Ingestion)	O	Decreased	O	O	O	O	O	*
Alkaline (Bacterial Decomposition)	O	Decreased	Decreased	False Positive	O	O	O	*
Acid (Ammonium Chloride Ingestion)	O	O	O	Decreased?	Decreased?	O	O	O
Ascorbic Acid (High Normal)	O	O	O	Decreased?	Decreased?	Decreased?	O	O
Ascorbic Acid (Therapeutically High)	O	O	Decreased	Decreased	Decreased	Decreased	O	Decreased?

* With this test, so many normal and abnormal urines gave positive results without added blood that the effect of various factors was impossible to determine.

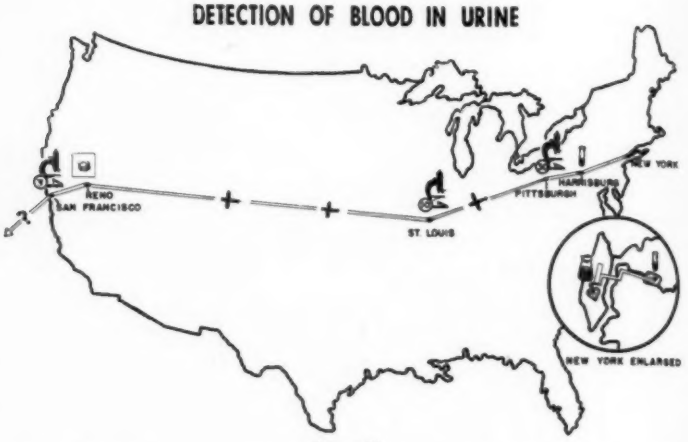

Figure V

The analogy of distances on a map gives the relation of sensitivity of various techniques for detecting blood in urine. If there were 100 per cent blood in the heart of New York City (Times Square) and if it became increasingly diluted with urine as it spread toward San Francisco then the following phenomena might be noted: gross blood detection 95% accurate at Central Park; benzidine dihydrochloride test barely positive at LaGuardia Airport; orthotolidin base trace at Harrisburg; microscopic examination 30 RBC/hpf at Pittsburgh and 10 RBC/hpf at St. Louis; Occultest barely positive at Reno; microscopic 3 RBC/hpf at San Francisco; Zwarenstein inderterminate due to positive reactions with normal urines.

are no longer detectable by microscopic examination.

Existing chemical tests for occult blood in urine also show extreme variation in sensitivity. The benzidine dihydrochloride test is not much more sensitive than visual recognition, while Zwarenstein's test is so sensitive that it reacts with as many as 25 per cent of urines collected from healthy subjects. The majority of chemical tests are influenced by urine variables which include specific gravity, ascorbic acid concentration and acidity or alkalinity.

Occultest is an interesting new chemical test for occult blood in urine which has advantages over both chemical tests and microscopic tests. The sensitivity of Occultest is quite high and compares with the sensitivity which is attained with microscopic examination. It has two advantages over microscopic examination in the detection of blood in urine. In the first place it is simple, rapid and requires no special equipment. Secondly, it is not influenced by hemolysis of red cells which occurs with some urines. Occultest also has advantages over other chemical tests. It is highly sensitive, yet does not react positively with urines

from healthy subjects. Occultest is not influenced by the several factors studied which change the sensitivity of most chemical tests.

The extreme sensitivity of Occultest makes it necessary that the test be carried out under scrupulously clean conditions. In the present study, the working surface employed was a sheet of clean white paper on which were placed the filter paper squares. This facilitates disposal of completed tests and also avoids possible contamination which might be contributed by extremely small residues of blood on table or bench surface.

SUMMARY

The sensitivity, efficiency and accuracy of various means of detecting blood in urine have been compared. Visual recognition of blood is useful and effective with large quantities of blood in urine, such as one part of blood in 125 parts of urine. Microscopic examination of sediment is capable of detecting quantities of blood as small as one part in 300,000 parts of urine, but hemolysis may occur with urines having low salt concentration or high pH. Chemical tests vary widely in sensitivity, primarily depending upon the exact procedure of the test. Chemical tests are influenced by variations in urine composition. Occultest, a new chemical test for occult blood in urine, has a sensitivity which compares with that achieved by microscopic examination and is not significantly influenced by urine variables.

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AN EVALUATION OF LYOPHILIZED PLASMA IN THE COAGULASE TESTS FOR MICROCOCCAL PATHOGENICITY*

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Since the first report of Loeb,[1] that pathogenic strains of staphylococci would coagulate blood plasma, the coagulase test has received increased acceptance as a means for differentiating such pathogenic from non-pathogenic forms. The tests for coagulase and hemolysis activities do not show complete correlation, but it would appear that there is a closer relationship between the pathogenicity and the coagulase test than between the pathogenicity and the hemolysing ability of the organism. As an example, Chapman, Behrens, Peters, and Curcio,[2] reported that in 690 of pathological strains of S. Aureus, 51.7% gave positive hemolysis, whereas 88% of these strains showed coagulase activity. Cruickshank[3] indicated that coagulase is present only in pathological strains of staphylococci—regardless of pigmentation —and suggested that the test be run routinely because of its ease of determination and constancy. In a later paper Chapman and his colleagues[4] stated that the coagulase test is the most reliable single test for the differentiation of the pathological from the non-pathological staphylococci. There is, however, no correlation between the amount or character of the coagulum and the degree of pathogenicity. The simultaneous determination of the hemolysin activity may give such an estimate. Further work by Fairbrother,[5] Moss, Squires, and Pitts,[6] and others substantially supported the importance of the test and led to certain general conclusions as to its reliability. It would appear that coagulase positive strains of staphylococci are pathogenic. A negative coagulase test does not, however, exclude pathogenicity. In other words, the coagulase test gave no false positives. The hemolysin test, in contrast, does yield some false positives, particularly in certain strains of S. Albus. Most recently, Jackson and his colleagues[7] recognized the dependability of the coagulase test in using it as a standard against which to evaluate the reliability of alpha-hemolysin production as a test for pathogenicity.

The basic method for performing this test was reported by Chapman, Behrens, Peters, and Curcio,[2] and is described in manuals of diagnostic bacteriology and clinical laboratory techniques. In brief, the test determines the ability of 0.5 ml. of a pure broth culture, or loopful of pure culture from an agar slant, to coagulate 0.5 ml. of fresh citrated plasma—either whole or appropriately diluted with saline.

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

The training of laboratory technologists is the primary interest of the author. Many of the students will eventually be working in small laboratories or physicians' offices where fresh citrated plasma, the use of which was emphasized by Chapman *et al.*,[8] may not be readily available. It therefore seemed of value to determine whether a commercially available lyophilized plasma standard could be used in this test. Accordingly, experiments were set up comparing the reaction of such a plasma with fresh citrated plasma. This diagnostic plasma is rigidly controlled as to its content of coagulation factors. It is used as the standard in prothrombin determinations and its stability and uniformity of response have been reported by Hodes[9] and by Oktavec and Smetana.[10, 11] If it could be used, as well, in the coagulase reaction it would avoid the necessity of keeping a special plasma preparation on hand for the coagulase tests.

Materials and Methods

Plasma: Two types of plasma were used in the experiment. The first was citrated plasma separated from freshly drawn normal human blood. The second was a commercially available lyophilized plasma* normally used as a prothrombin time standard. The material was reconstituted according to the directions by adding 0.5 ml. of distilled water (pH 6.6) to each vial of dried plasma. The contents of several vials were pooled. Saline dilutions of both the fresh citrated plasma and diagnostic plasma were made according to the scheme shown in Table I.

TABLE I
Dilutions of Plasmas

Dilution	1:1	1:2	1:3	1:4
Plasma, ml.	6	3	2	1.5
0.85% saline, ml.	0	3	4	4.5

Organism: The micrococci used in the coagulase tests were obtained from two sources—from the stock culture collection of the Laboratory of Microbiology of the University of Pennsylvania, and from freshly isolated pathological material sent to the Bacteriology Section of the William Pepper Laboratory, Hospital of the University of Pennsylvania, kindly supplied through the courtesy of Mr. William Brandes. The cultures were grown for 18 hours at 37° C in freshly prepared Beef Heart Infusion Broth or upon Beef Heart Infusion Agar. Material from the Agar cultures was suspended in a small amount of saline prior to testing.

Method: The method of Chapman, Behrens, Peters and Curcio[2] as outlined in *Diagnostic Bacteriology*, Schaub and Foley, 4th ed. was used. The tests were set up in 12 x 75 mm. test tubes. Four

* Diagnostic Plasma, Lot No. 062051, supplied by Warner-Chilcott Laboratories, New York City.

TABLE II

Comparison of coagulase reaction of dilutions of reconstituted diagnostic plasma, "D," and citrated fresh human plasma, "C," tested with stock cultures of micrococci and with freshly isolated cultures of micrococci obtained from pathological material. Reaction mixture was 0.5 ml. of plasma dilution and 0.5 ml. of 18 hours broth culture.

Plasma dilution	1:1								1:2								1:3								1:4							
Time of observation, minutes	30		60		120		180		30		60		120		180		30		60		120		180		30		60		120		180	
Type of plasma	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C
Culture																																
M. pyog. aureus*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
M. roseus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
M. pyog. species	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
P. L. 7747	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P. L. 7749	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
P. L. 7857	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
P. L. 7863	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+
P. L. 7866	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+

Cultures labeled P.L. were from the William Pepper Laboratory, Hospital of the University of Pennsylvania.

Column "D" indicates Diagnostic Plasma; column "C" indicates fresh citrated plasam.

* There was poor growth in the culture of this organism.

rows of test tubes were placed in a wire rack and labeled with the proper plasma dilution and the number of the culture to be tested. Each tube received 0.5 ml. of the proper dilution of plasma. Each tube then received 0.5 ml. of an 18 hour broth culture of the designated organism or a loopful of the saline suspension of the culture from the agar slant. The tubes were then placed in a water bath at 37° C. and observed for coagulation at 30, 60, 120 and 180 minutes.

Results

A preliminary check using the diagnostic plasma and stock cultures indicated that coagulation took place satisfactorily in the reconstituted plasma in saline dilutions as high as 1:4. A comparison was then made between the reactivity of the reconstituted diagnostic plasma and the citrated normal fresh plasma at the dilutions previously mentioned (See Table I). Since it was felt that there might be some alteration in the level of coagulase activity in the stock cultures due to the development of varients, five freshly isolated cultures of micrococci obtained from pathological material were also tested for activity. These latter cultures more closely approximate those which would be used in the routine performance of the test. The results are shown in Table II.

As can be seen, there was complete agreement between the results with the diagnostic plasma and with the fresh citrated human plasma. In all tubes containing coagulase positive organisms coagulation was observed within 60 minutes. It must be remembered that the degree or character of the coagulum is not a factor in the interpretation of the results—any coagulation within 3 hours is a positive test. Both the diagnostic plasma and the fresh plasma could be diluted 1:4 with 0.85% saline and still give results identical with whole plasma.

Tests were also run using organisms cultured on agar slants. The culture was removed from the agar and suspended in a small amount of 0.85% saline. 0.5 ml of this suspension was added to 0.5 ml of plasma dilution in the same manner as above. There was complete correlation between the results with the broth and agar cultures, in the fresh and diagnostic plasma. However, the reaction with the slant cultures seemed to be somewhat slower even though the bacterial suspensions appeared heavier than the bacterial growth in the broth cultures. This slight delay may be explained by the possibility that coagulase is more readily available in the broth cultures than in the saline suspensions of the bacteria.

Discussion and Conclusions

The importance of using fresh plasma in the coagulase reaction was stressed by Chapman.[8] From the results reported here it is

evident that this commercially available lyophilized plasma standard gives comparable results. While the mechanism of the coagulase reaction is not completely understood, it seems probable that the blood constituents essential to the coagulase reaction are identical with substances reacting in the normal coagulation process. Miale[12] has suggested that the plasma constituent acted on by staphylo-coagulase is a globulin—probably identical with a normal coagulation factor, thromboplastinogen. This commercially available diagnostic plasma, since it was originally developed as a standard plasma in the determination of prothrombin time, is carefully standardized as to its content of coagulation factors. This uniformity should be equally important in the reproducibility of the coagulase reaction.

Summary

1. A commercially available dried plasma, when reconstituted according to direction gives results identical to those obtained with citrated freshly drawn normal human plasma in the coagulase test for the pathogenicity of staphylococci.

2. This plasma gave equally good results when diluted 1:4 with 0.85% saline.

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USE OF THE PROTHROMBIN CONSUMPTION TIME IN THE DIAGNOSIS OF HEMORRHAGIC CONDITIONS*

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Prior to 1930 a study of any hemorrhagic condition was limited to the Lee-White coagulation time, the bleeding time and the platelet count. A diagnosis of hemophilia was made whenever the coagulation time was prolonged and thrombocytopenia when a reduced platelet count and a prolonged bleeding time were obtained.

With the introduction of the one-stage prothrombin time by Quick[1] in 1935, it was possible for the first time to distinguish two conditions giving a prolonged coagulation time. In one, namely, hemophilia, it was established that the prothrombin concentration was normal, whereas in the second, the prothrombin time was prolonged and, therefore, the condition was named "hypoprothrombinemia." The prothrombin time is based on the concept that when a constant amount of calcium and a standardized preparation of thromboplastic reagent is added to oxalated plasma, the clotting time becomes a measure of the prothrombin concentration. It was found that rabbit-brain dehydrated with acetone provided a preparation which was not only highly potent but was remarkably constant in its activity. Since it was found later that other factors besides prothrombin when diminished prolonged the prothrombin time, it was necessary to use the term hypoprothrombinemia as a general name for several conditions and to interpret the prothrombin time as a measurement of prothrombic activity.[2]

After the establishment of methods to measure prothrombic activity, the task still remained to find means for measuring the thromboplastin which presumably was generated during the clotting of the blood. A successful method was developed by Quick[3] on the basis of measuring the prothrombin that remained after blood had been clotted under standardized conditions. By means of this test, he found that almost all of the prothrombin in hemophilia blood remained after such blood clotted. In this study, evidence was found that in hemophilia thromboplastin did not form because of a deficiency of a plasma protein which he named "thromboplastinogen."

The prothrombin consumption time is a means of indirectly measuring the amount of available thromboplastin of the blood. The circulating blood never contains active thromboplastin but rather contains the precursors required to form thromboplastin. In the first phase of the coagulation reaction, as seen in Figure 1, the platelet factor interacts with the thromboplastinogen com-

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956. Scientific Products Foundation—Hematology, 3rd Award.

plex to form thromboplastin. Whenever thromboplastin is available, the second phase of the reaction proceeds so that prothrombin is converted to thrombin. Normally a little prothrombin remains after the entire reaction has reached an equilibrium and the prothombin time of serum obtained from normal blood is seventeen seconds or longer. Whenever one of the factors from the first phase is deficient, it limits the amount of thromboplastin formed thus reducing the amount of prothrombin which is converted to thrombin. The serum resulting under such a condition contains more prothrombin than normal and as a result the prothrombin time will be less than seventeen seconds. In severe deficiencies practically all of the prothrombin remains and a time of eight seconds results.

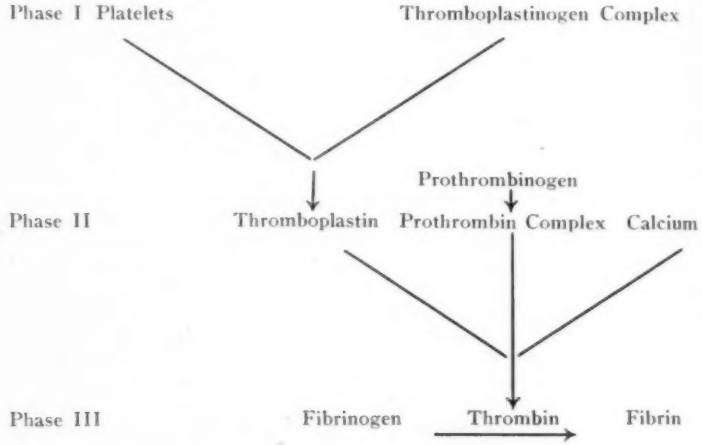

Figure 1. Coagulation Theory of Dr. A. J. Quick. Constituents of thromboplastinogen complex: thromboplastinogen, plasma thromboplastic component (PTC), and plasma thromboplastin antecedent (PTA).
Constituents of prothrombin complex: prothrombin, labile factor, and stable factor.

At this point we can see that the prothrombin consumption will be short whether platelets or one of the factors from the thromboplastinogen complex is deficient. By adding normal platelets to blood before coagulation occurs, the test is made more specific since the prothrombin consumption time can then be attributed specifically to a deficiency of one of the factors which constitutes the thromboplastinogen complex.

The isolation of platelets by differential centrifugation and

washing them free of plasma is time consuming. It was therefore fortunate to find that rabbit brain thromboplastin, if incubated at 60° C instead of 50° C, serves as a substitute for the platelet factor.[4] Another advantage to the use of this heated thromboplastin is that it gives more consistent results with the prothrombin consumption time in mild hemophilia. This latter action is probably due to the presence of an inhibitor which depresses the activity of a clotting factor which is released from the erythrocytes when they are hemolyzed. Thus by means of the regular prothrombin consumption time and the modified test with 60° heated thromboplastin added to the blood before coagulation, the diagnosis of even mild hemophilia can be made. The modification of the prothrombin consumption time using the heated thromboplastin is referred to as the thromboplastinogen activity time (TAT). Values obtained by the two tests are essentially the same except that in mild hemophilia, it is not unusual to find that the thromboplastinogen activity time gives a much shorter value than the original prothrombin consumption time.

METHODS

1. *Lee-White Coagulation Time:*[5]

Blood is drawn by careful venipuncture with silicone-coated needle and syringe. One cc. of blood is introduced into each of two clean dry glass test tubes (13 × 100) and kept in a waterbath at 37° C. At the end of five minutes the tubes are gently tilted to test for clotting. This process is repeated every 30 seconds until the tubes can be inverted without any flow of blood. If coagulation has not taken place within 15 minutes the tubes are checked every 2 to 3 minutes rather than at 30 second intervals.

2. *Prothrombin Consumption Time:*[6]

Blood is collected and the Lee-White coagulation time is performed. Fifteen minutes after the clot has formed, the tubes are centrifuged at 2000 rpm for one minute. The tubes are returned to the waterbath for incubation until one hour after coagulation has taken place. (Note: Do not remove the serum from the clot until the end of the incubation time.)

At the end of the hour incubation, the serum is assayed for prothrombin by combining:

0.1 cc. deprothrombinized rabbit plasma
0.1 cc. thromboplastin, rabbit brain
0.1 cc. calcium chloride (0.02M)

and then blowing 0.1 cc. of serum into the mixture and simultaneously starting a stopwatch. The end-point is the appearance of fibrin.

3. *Thromboplastinogen Activity Time (TAT):*[4]

Acetone-dehydrated rabbit brain thromboplastin (200 mg. per 5 cc. of physiological saline) is incubated at 60° C for 20 minutes.

For the test, 0.05 cc. of the thromboplastin is pipetted into a clean dry glass test tube (13 × 100). Blood is drawn by venipuncture and 2 cc. of blood is added to the thromboplastin and mixed by inversion. The tube is placed in a waterbath at 37° C and the time for coagulation is noted. Fifteen minutes after clotting the tube is centrifuged for one minute at 2000 rpm and then returned to the waterbath until one hour after coagulation. At this time the serum is assayed for prothrombin as in the prothrombin consumption time.

4. *Platelet Count:*[6]

Blood is drawn by venipuncture using silicone-coated needle and syringe and then 1 cc. of blood is added to 0.5 cc. of 3.8% sodium citrate in a small silicone-coated test tube. If not used immediately this mixture should be kept in the refrigerator. However, it is advisable to complete the count within two hours. After carefully mixing the blood with the citrate, the diluted blood is drawn into a red cell pipette to the 0.5 mark and then further diluted with citrate to the 101 mark. The pipette is shaken for five minutes and a standard counting chamber is then filled as for a blood count. The chamber is then placed in a petri dish with a moist filter paper to allow the count to settle without drying. After fifteen minutes the platelets in a sq. mm. (entire red cell area) are counted.

Calculations: Platelets/sq. mm. $\times \frac{3}{2} \times 200 \times 10 =$ Platelets/cu. mm.

TABLE 1
Results on patients diagnosed as Hemophilia.

	Lee-White Time in Min.	Prothrombin Consumption Time in Sec.	TAT Time in Sec.
Normal	5—10	17 or more	17 or more
T.P.	90—105	9	9
D.S.	60—65	8	8
B.L.	20—25	8½	8½
A.F.	14—18	11—11½	10
J.P.	10½—12	13½	13
B.B.	8—9	14	9
M.F.	7½—8	19	9

RESULTS

In our laboratory extensive studies are carried out on all types of hemorrhagic conditions and therefore I have access to hemophiliacs of varying degree of severity. In Table 1 are some typical results from a representative group of hemophiliacs. Generally, if the Lee-White coagulation time is 20 minutes or longer there is no consumption of prothrombin. In patients with coagu-

lation times that approach normal, some consumption of prothrombin can be measured. In fact, as will be observed from the table, a fairly normal prothrombin consumption is sometimes obtained in mild hemophiliacs who have a normal clotting time. In such cases, however, the thromboplastinogen activity time remains in the hemophilic range, namely, between 8 and 13 seconds. The erroneous results obtained with the unmodified prothrombin consumption time can often be traced to a minute amount of hemolysis which liberates a clotting factor from the erythrocytes. The correctness of this conclusion is known since when platelet-rich plasma is clotted instead of the whole blood, a short prothrombin consumption time is obtained.[6]

In contrast to the results obtained in cases of hemophilia, in Table 2 are the results from several cases of thrombocytopenia and a case of thrombasthenia. All of the coagulation times are normal which is a result commonly found in uncomplicated thrombocytopenia. With platelet counts ranging from 18,000 to 54,000 all of the prothrombin consumption times were abnormal. With the thromboplastinogen activity time, however, good correction is obtained which is to be expected, since the thromboplastin heated to 60° C is a satisfactory substitute for platelets.

TABLE 2
Results on patients diagnosed as Thrombocytopenia and Thrombasthenia.

	Lee-White Time in Min.	Prothrombin Consumption Time in Sec.	TAT Time in Sec.	Platelets Count/cu.m
Normal	5—10	17 or more	17 or more	100,000—400,000
M.F.	7	11—12	27½	21,000
M.F.	7½—8	12	20	18,000
M.G.	8	13½	27	54,000
M.Z.	8½—9	11½—12	24	21,000
M.H.	6½	14	18½	258,000

In the case of M. H., who has thrombasthenia, we see that although the quantity of platelets is normal, 258,000, qualitatively they are deficient in the factor necessary for thromboplastin formation since the prothrombin consumption time is only 14 seconds. The correction obtained with the thromboplastinogen activity time clearly shows that the plasma thromboplastinogen complex factors are normal.

DISCUSSION

When blood is taken from its natural environment and placed in a glass test tube it requires only a minute amount of thrombin to give a solid clot. The rate at which this thrombin is formed will determine the Lee-White coagulation time. In this manner, only severe deficiencies of substances such as prothrombin or

thromboplastinogen will cause sufficient delay in the formation of thrombin to give a prolonged time. Minute amounts of tissue juice will be sufficient contamination to change a prolonged time to normal.

Since in the first and second phase of the coagulation reaction the substances interact on a stoichiometric basis, as long as the physiological balance is maintained, tests such as the prothrombin time, the prothrombin consumption time and the TAT are sensitive to small fluctuations in the concentration of the various factors. Therefore, these are specialized methods to use in diagnosing hemorrhagic conditions.

At the present time many of the mild bleeders are being diagnosed only after they have had severe difficulties following surgery and required numerous transfusions to control the hemorrhage. Although the physician is responsible for the treat ment of the patient, the laboratory has a responsibility to utilize available tests of sufficient sensitivity to aid in the diagnosis and treatment of the hemorrhagic condition.

The use of the bleeding time, the prothrombin time, the prothrombin consumption time, and the thromboplastinogen activity time in studying a patient having a history of family bleeding or one who is subject to easy bruising or bleeding after minor surgical procedures as circumcision or tooth extraction, will aid the surgeon in determining if the patient is a safe surgical risk.

SUMMARY

Studies on cases of hemophilia, thrombocytopenia and a thrombasthenia were made. The procedures used were the platelet count, the Lee-White coagulation time, the prothrombin consumption time and the more recently developed thromboplastinogen activity time. Note: All of the reagents used in the procedures described are available commercially.

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HORMONE DETERMINATIONS IN THE CLINICAL LABORATORY*

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During the past few years a new field of determinations has developed within the clinical laboratory. Many of these determinations have been the results of investigations in general physiological chemistry concerned with the function and chemistry of the endocrine system. Because of their research background there is a certain degree of apprehension with respect to the relative ease and feasibility of the determinations. Although it is impossible in a short review to adequately outline the exact procedures, it is possible to indicate the more useful tests and the easiest methods for performing them. In this review, I shall endeavor to cover tests for thyroid function, adrenal cortex function, gonadal and placental function and certain pituitary hormones.

Modern methods for determining thyroid function are based on the determination of protein bound iodine and the uptake, distribution and excretion of radioactive iodine. It is important to note that these tests are not competitive but rather complementary, since they determine different aspects of thyroid function. The chemical test for protein bound iodine is essentially a measurement of the thyroid hormone, thyroxine, at the serum level. Radioactive iodine uptake measures the rate at which the thyroid accumulates and excretes iodine. Similiarly, measurement of radioactive iodine in urine indicates the rate at which iodine is excreted from the body. The radioactive protein bound iodine measurement is a ratio of precipitable radioactive iodine to total radioactive iodine in serum. It is unfortunate that the term "radioactive PBI" has been employed since it serves to confuse a ratio measurement with an absolute measurement. That is to say, the radioactive PBI does not measure the level of thyroxine in serum as does the chemical PBI. Three methods of determining the chemical PBI are available. These are the dry ashing technique developed by Salter[1] and modified by Barker,[2] the perchloric acid technique of Loeffler & Zak[3] and the acid-distillation technique developed by Chaney[4] and simplified by Moran.[5] From personal experience I recommend the acid-distillation technique for its virtues of simplicity, speed, accuracy and reproducibility of results.

In determining adrenal cortex function three well-established procedures and two relatively new procedures are available for

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routine use. The adrenal glands consist of two distinct tissues in close apposition; the medulla, which produces epinephrine and norepinephrine and the cortex which produces the steroids. To date, approximately 60 steroids have been isolated in crystalline form. However, the great majority of these are physiologically inactive so far as is known. Since cortex tissue is embryologically related to gonadal tissue, many of the steroids have androgenic qualities and are responsible for secondary sex characteristics. The psysiologically active steroids are essential in maintaining electrolyte balance, protein and carbohydrate metabolism, in providing resistance to stress and some bear a causal relationship to the collagen diseases. The underproduction of steroids, due to progressive destruction of the cortex, results in Addison's disease. The overproduction of the steroids responsible for carbohydrate metabolism results in Cushing's syndrome. The overproduction of adrenal androgens leads to the adrenogenital syndrome with resultant virilism, hirsutism or precocious masculinization. Except in relatively infrequent and advanced cases, the diagnosis of adrenal cortex dysfunction is extremely difficult from clinical signs and symptoms. Adding to the problem is the fact that dysfunction may be of varying degree and selectivity. Since the pituitary gland is responsible for the stimulation of the adrenal cortex through the adrenocorticotrophic hormone (ACTH), dysfunction of the pituitary will also cause dysfunction of the adrenal cortex. To bring some order out of this apparent confusion we now have avialable the five tests previously mentioned.

All of these five determinations depend on extraction of the desired components by means of suitable solvents at proper pH levels and subsequent color development with specific reagents. The best known of these is the procedure for neutral 17-ketosteroids. Measurement of 17-ketosteroid output is a biochemical index of testicular and adrenocortical activity. The method I prefer was developed at McGill University Clinic and is outlined in "Practical Physiological Chemistry" by Hawk, Oser and Summerson.[6] However, for the final reading I extract the color with redistilled amyl acetate (as recommended by Moran in a private communication) and take a single reading at 530 m.u.

Closely allied to the 17-ketosteroid determination is the test for 17-ketogenic steroids, developed by Norymberski.[7] An excellent account of his method is available in the December 1952 issue of Nature. The 17-Ketogenic steroids are those which can be oxidized with sodium bismutate to the 17-Ketosteroid group, and are presumed to be the fore-runners of 17-ketosteroids. After oxidation with sodium bismuthate in the presence of glacial acetic acid, the urine sample is treated in the same fashion as a 17-ketosteroid determination. This test is particularly useful in

determining the corticosteroids responsible for maintaining carbohydrate and protein metabolism.

In the differentiation of adrenal cortical tumor from hyperplasia the test for dehydroisoandrosterone is most useful. The most widely used method is the one introduced by Allen although for reasons of specifiicity and brevity I prefer the method of Landau[8] and his co-workers. The method is detailed in the May 1951 issue of Endocrinology.

The fourth test for adrenocortical activity is the determination of 17-hydroxycorticosteroids. This is essentially a test for cortisone and hydrocortisone and is very useful in establishing diagnosis for Cushing's Syndrome. It has equally important usefulness in differential diagnosis. The test is among the simplest of the steroid determinations. The method I prefer is based upon Reddy's test employing the Porter-Silber color reaction. Among the best modifications is the one presented by Miss Kathleen Clayson[9] of the University of Minnesota at the ASMT convention in New Orleans in June 1955. Although the color development reported by Miss Clayson is somewhat different from the method I personally employ, for reasons of simplicity I recommend the method suggested by Miss Clayson.

The fifth important test is the determination of 11-oxycorticosteroids. This is a specific test for corticosterone and 11-dehydrocorticosterone which possess both electrolyte balance and carbohydrate metabolism properties. However, this test is the most difficult and tedious of the group and should not be attempted until the previous four have been mastered. The method I prefer is the conversion of the 11-oxycorticosteroids to formaldehydrogenic steroids. The best account is that of Corcoran[10] and co-workers which appeared in the November 1948 issue of the Journal of Laboratory and Clinical Medicine. In the modification suggested to me by Moran, I employ sodium bismuthate instead of periodic acid for the oxidation.

In determination of gonadal and placental hormones the determination of estrogens and pregnandiol are required. For male gonadal activity the determination of 17-ketosteroids and dehydroisoandrosterone is required. At present, the only methods for estrogen applicable to the clinical laboratory involve flourometry. Although certain color reactions, such as the Kober phenylsulfonic acid and Ciocalteu phenol reagent are available, their use in the minute determinations required make it virtually impossible to obtain representative determinations. The flourometric method which I recommend is that of Finkelstein[11] presented January 1948 in the proceedings of the Society for Experimental Biology and Medicine. It is only fair to note that at best the method is difficult and tedious.

Fortunately, the determination for pregnandiol is not particu-

larly difficult, although it is somewhat lengthy, requiring about 2 hours of the analysts time. The test is best performed by Sommerville's[12] method which appeared in the March 1948 Journal of Endocrinology. This method is recommended since it is able to recover very low concentrations of pregnandiol. The determination of pregnandiol is an accurate measurement of the production of progesterone by the corpus luteum of the ovary and, of course, by the placenta during gestation.

Tests for the pituitary hormones FSH and LH are bio-assay procedures. FSH is the follicular stimulating hormone and LH is the luteinizing hormone. Only the former can be readily adapted to the clinical laboratory since LH determinations require hypophysectomized rats. The FSH determination I employ measures the increase in uterine weight to gross weight ratio in standardized immature female rats. This method is based upon Jungck's technique presented in January 1947 in the Journal of Clinical Endocrinology. However, the method is fairly complicated and requires considerable experience. A more suitable method for the clinical laboratory is outlined in "Laboratory Aids in Endocrine Diagnosis" by Escamilla[13] and is a modification of Dekanski's work and Bradbury's work. In this method immature female mice are employed and the hormones are concentrated by absorption on kaolin.

The development of important tests for endocrine function is an important advance in clinical laboratory science. It is my hope that this brief review of some of these tests and the recommendations for suitable methods will assist in that advance.

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HEALTH HAZARDS IN THE LABORATORY

Laboratory safety, like highway safety, is a state of mind. The medical technologist and the laboratory technician must be aware of the possibilities of danger which surround them at all times and learn to live with them with intelligence and without fear. "An ounce of prevention is worth a pound of cure" is doubly true in the laboratory. A carelessly discarded match can cause a costly fire and even endanger life.

I think the bacteriology angle has been most ably handled by the group at Camp Detrick.

In chemistry care usually is taken about large and obvious hazards but some of the smaller ones can be equally damaging.

1. Never store strong acids or corrosive chemicals on a shelf above eye level.
2. Label all solutions *correctly*.
3. Mark all *Poison* substances as such and observe prescribed precautions.
4. Dispose of chemical wastes with care, especially those containing cyanide.
5. Be sure to have adequate ventilation when using carbon tetrachloride and similar substances.
6. Fire polish sharp glass edges.
7. Store gas tanks with care, large tanks should be firmly attached in a carrier or anchored with a chain.
8. Keep the number of extension cords to a minimum and be careful of frayed cord and defective sockets.
9. Do not push the lighted bunsen burner under the shelf! Almost every laboratory shelf has a scorched spot. (There is a new burner on the market now which would eliminate this hazard.) See that the rubber tubing on the bunsen burner is in good condition.
10. Be familiar with first aid technics and have materials easily available to all workers in the laboratory. Know where the fire extinguisher is and how to use it.

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THE LYMPH NODE AND THE PROCEDURE IN PROCESSING IT FOR MICROSCOPIC DIAGNOSIS*

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There are some types of tissue which demand special care in order to achieve a successful preparation of the tissue for microscopic study. Lymphatic tissue appears to show great distortion if not processed carefully. Many technical factors should be considered but unless one understands the histological picture of the specimen, it is difficult to realize the importance of good technical care.

The lymph node is composed of lymphatic tissue with a framework of fibrous tissue. The framework consists of a capsule and of trabeculae which pass at intervals inwards from the capsule and after transversing the cortex of the gland, divide and reunite with one another to form a network in the medulla. The capsule and the trabeculae contain muscular tissue. The glandular substance is composed of a fine reticulum with the meshes thickly occupied by lymphocytes.

DISCUSSION

Poor fixation, shrinkage and brittleness of the specimen are the prime reasons for poor sections. A poor quality of staining in the center of the lymph node generally means poor fixation. Shrinkage occurs in the meshwork around the glandular substance and brittleness is very often seen in the muscular tissue.

The smaller the lymph node, the more attention it must be given because the dehydration period for it will be shorter than for a larger one. When metastasis is present, the technical difficulties are less because the tumor increases the cohesiveness of the cells. The processing of biopsy material gives cause for concern. A diagnosis depends upon the microscopic study of the tissue and if final results are not satisfactory, additional biopsy tissue may not be available and a diagnosis cannot be made. It has been proven that lymphatic tissue needs longer fixations than other types. Bouin's fixation is considered satisfactory for the study of the cellular structure but it will cause brittleness. The length of time required for the fixation varies, depending upon the size of the specimen.

Buffered formalin has proved the best fixative. Ordinary ten per cent formalin is frequently used but there are benefits to be derived from using buffered formalin. For example, pigment often seen in formalin-fixed tissue is not found when buffered formalin is used.

All the rules may have been followed regarding fixation, but

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956. Scientific Products Foundation—Histology, First Award.

improper dehydration may still cause dire results. Every institution has its preferred process of dehhydration and probably believes its procedure the best because, in general, it lends itself to most types of tissue.

Through experimentation it has been found that slow penetration of the dehydrating fluids gives satisfactory results. Starting with a low percentage of alcohol and gradually raising it to 100% is an effective procedure.

Acetone and dioxane are two dehydrating fluids to which lymph nodes must not be subjected. They are too rapid and cause a great deal of brittleness and shrinkage. Ethyl alcohol is advisable. Propyl alcohol 99% may be used with success. It is a graded alcohol in itself, in that it will mix with water and also with any clearing agent.

The time required for dehydration depends upon the size of the tissue. This is one of the reasons for not including lymph nodes in the daily procedures for surgical specimens. Most tissue specimens are in the clearing agent for at least one hour and in two changes of paraffin for one hour. This is too long for most lymph nodes.

Clearing agents are always a topic of discussion. One laboratory maintains that xylol is the best; another uses benzene. By experimentation it has been shown that zylol makes lymph nodes brittle. Benzene proved somewhat better. Terpineol and chloroform, half and half, for one change followed by chloroform gives good results.

The important considerations with respect to the embedding medium are the temperature at which it is kept and the period of time the tissue is subjected to it. No lymphatic tissue needs more than half an hour in the first paraffin and an hour in the second. If the specimen is small, fifteen minutes in the first and half an hour in the second will be sufficient. The temperature must be kept below 60 degrees, preferably around 56 to 58 degrees.

When embedding has been reached, one must be certain there is no undue pressure on placing the lymph node in the paraffin. The nodes are of soft consistency and distortion will appear following pressure on the specimen.

A very sharp knife free of nicks is necessary for sectioning. There are times when a tissue will have some particles of calcium deposit in it. When this is found to be true, place the block in the machine with the hard spots farther away from the edge of the knife so that on cutting, the knife does not pass through this area first. In this way there will be areas left that will show no distortion. It is advisable to ice the knife but not the block when cutting this type of tissue. Slow movements in sectioning are desirable. Sections cut slowly do not show a crowding of the cells as in the case when fast cutting is done.

The water bath should not be allowed to overheat. Overheating will cause spreading of the meshwork and misplacement of the cells.

Ten minutes only should be allowed for melting off the paraffin from the slides if a paraffin oven is used for this purpose.

The staining of the lymph node should be watched microscopically. There is a tendency in laboratoreis to pass sections routinely through solutions from start to finish without microscopic examination. The lymphocytes have the tendency to stain darkly and this has to be watched to insure that the nuclei of the cell is well differentiated.

RESULT

The above procedures may sound very time-consuming and complicated, but the satisfaction of seeing a well-prepared slide is worth the trouble involved. Diagnosis may have been delayed but, on the other hand, uncertain diagnosis might have caused the patient and the doctor a great deal of anxiety.

METHOD

Fixative	Buffered formalin
Formalin (strong) 40%	18 cc.
Tap water	162 cc.
Acid Sodium Phosphate Monobasic	0.92 gms.
Anhydrous Sodium Phosphate Diabasic	1.17 gms.

Time of fixation—Overnight is possible

Rinse the tissue off in running water for 15 to 20 minutes. Place the capsule containing the tissue into the basket and connect to the rotator on the autotechnicon. Allow to rotate in the following fluids:

80% Alcohol	1 hr.
95% Alcohol	1 hr.
95% Alcohol	½ hr.
Abs. Alc.	1 hr.
Abs. Alc.	1 hr.
Abs. Alc.	1 hr.
(if nodes are small, cut time to ½ hr.)	
Chloroform and Terpineol, half and half	½ hr.
Chloroform	½ hr.
Tissuemat No. 1 (Temperature not over 60 degrees)	½ hr.
Tissuemat No. 2 (Temperature not over 60 degrees)	1 hr.

The above procedure takes over eight hours to complete. If necessary, the tissue may be placed in the second paraffin (in a small jar) and left outside the oven and allowed to harden. In the morning, the jar is placed in the oven to melt the paraffin. The tissue is placed in fresh paraffin for 15 minutes. It is then embedded. The above procedure may be started at 8:00 A.M. By 4:00 P.M. the tissue can be cut. If the lymph node is large, it must be sectioned in half before processing.

SUMMARY

The points to take into consideration if this method is followed may be briefly stated:

1. Good fixation
2. Slow dehydration
3. Control of temperature at all times
4. Slow cutting of sections
5. Microscopically controlled staining

ACKNOWLEDGMENTS

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MEDICAL TECHNOLOGIST PRAYER

O GOD, Who, by calling us to the vocation of a medical technologist, hast placed upon us the obligation of being a constant help in the scientific care of the sick, grant us by Thy divine light a deep insight into the serious responsibilities of our tasks. By Thy divine wisdom awaken in us a growing zeal and determination to increase our knowledge of how to search for the underlying causes of sickness and disease; how to recognize the evidence of physical changes; how to make important chemical analyses, and other valuable tests so helpful in caring for the sick. By Thy divine love permit us in this way to share with those who directly care for the sick, that thus we may be constantly working through the Eternal Physician, Christ, Our Lord. Amen.

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THE HUMANISTIC DISCIPLINES AND SCIENTIFIC THINKING*

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Foreword

With the increasing demand for well-trained, highly specialized medical technologists, the problem of adequate college preparation has become a matter of concern to medical educators. They are faced with two possibilities, either to provide a strictly vocational curriculum or to compliment the professional curriculum by including the study of the humanistic disciplines.

It is my opinion that the latter statement contains the solution of the problem. And it is in defense of this argument that I attempt to present the case for liberal education in university curricula for students of the medical specialties.

The subject of this paper should be of interest to the medical technologist insofar as the progress of her profession is her responsibility. The success of her career will eventually be determined by the educational standards established during these early years of growth and development.

This discussion is not intended to be scientific nor philosophic, but rather to acquaint the reader with a problem common to each of us entering a profession dedicated to the service of mankind.

A Medical Technologist

It is evident from the start that anyone who attempts to write a complete and detailed study of the place of the humanistic disciplines in scientific thought is confronted with the proper selection of factual material pertinent to such a brief survey. The purpose, therefore, of this paper is to present a few basic ideas in regard to the place of the humanistic disciplines, which will be defined briefly to mean those academic disciplines primarily designed to lay a solid foundation for the balanced cultural education of the human personality, in a modern world saturated with a demand for purely scientific thinkers.

To many educators there is no doubt concerning the sovereign position of the humanistic disciplines in modern education. A strong substructure is requisite to erect a tower of scientific study. But to accomplish this task, the objectives of liberal education must be directed toward the education of the whole person. That is, make him a true man of character, a person capable of leading a distinctly human life of reason.

If we are to fully understand the importance of the humanistic disciplines, we must establish their role as a foundation for lib-

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956. Scientific Products Foundation—Professional, First Award.

eral education. The question arises: what is a liberal education? Why is it said to be of such importance? And if it is worthy of attainment, how does one attend to educate himself liberally? These questions demand our attention.

Someone once said that before great answers can be given, great questions must first be asked. This is true of the questions proposed. The what, why and the how of a liberal arts education.

What is a liberal education? In his recent book, *The Art of Getting a Liberal Education*[1] Mortimer Adler defines it by stating that, "It (a liberal education) enriches us." Perhaps this is an all inclusive idea if one can really grasp the extent of its content. Certainly a truly liberal arts education requires universal knowledge, and nothing is more satisfying to man than to recognize that knowledge, the simplest and the profoundest of human experiences, is one and true. Aristotle in the earliest centuries of philosophic thought said that man, through knowledge, can become all things. That is, each man's life is enriched through knowledge.

Others have referred to a liberal education as a "smattering of nothing." The misconception of meaning in this statement is evident when we see that in addition to requiring broad general knowledge, a liberal education also includes a well-defined study of one definite phase of universal knowledge. The truly liberally educated man does not compartmentalize his knowledge into unrelated courses, but rather is fully aware of the relationship of one subject with another. Liberal education concentrates on many aspects of education and is not just concerned with one department or period of life. Instead, it attempts to give an understanding of all things in their proper proportions. Its proper study is life, the completeness of life.

A liberal education is not acquired in a day or even after four years of college. It is a life-long process which actually never ends because it is never completed. It is not just academic nor is it necessarily found in books or schools or teachers. Indeed, if the intellectual atmosphere of a university is to provide a liberal education and if the truth of knowledge is to be its aim, then it must be amplified and given substance in the world of experience.

After all a book is just a summary of the thoughts and experiences, real or imaginary, which an author puts on paper. But where did he find these thoughts? To really know one must seek the site of knowledge whether it be in the petal of a flower, in the sound of a beggar's plea, in the shout of war, the wonder of an ancient ruin, the inside of a church, in listening silently to a great piece of music, or in talking to strange people in

[1] Adler, Mortimer J., *How to Read a Book* or *The Art of Getting a Liberal Education*. Edition 22, New York, Simon and Schuster, 1940.

strange tongues in strange lands. Surely a liberal education, then, is not just academic.

Again a liberal education does not look only to the future, nor does it look only to the past, nor is it preoccupied with the present. It understands the time of all three. It grants that man should understand the heritage of the past, and because it knows man's hopes and ambitions it gives him a new vision of what to see in the future. Concerning everyday problems, a liberal education shows one how to work better, how to study deeper, and how to enjoy his leisure.

Its goals include integrity of spirit, the capacity for intellectual growth, the ability of man to foresee and rise, as it were, to a new situation or occasion. It creates in a society such as ours, men who are capable of discharging the responsibilities of free men. It cultivates and disciplines the minds of men to make them free in order that they may act free. We might add that to think correctly and freely and have all of the necessary knowledge to make a rational judgment is the object of a liberal education. For no one can really understand himself, and his world, can really know truth and beauty, can truly work toward a goal unless his mind is free from ignorance, unless his imagination is wakened and unless he has a disciplined reason.

The objectives of a liberal education are many, in fact too many to be discussed here. Nevertheless the objectives mentioned are the tenets of the humanistic disciplines, and the liberal education tends to assist the individual to reach these objectives.

How does one go about acquiring a liberal education? Academically this is accomplished first by training the student in the accurate use of his own language, to communicate his thought to others. Secondly, the student must be taught factual knowledge of the physical universe, the laws of societies in general, and his individual role in society. Training of students in correct thinking and proper judgment in issues of a social, moral or religious nature must be included. And lastly, we must encourage a new "intellectualism" in our society for those who desire the attainment of higher perspectives.

In this regard a liberal educator must consider the manner in which he will teach the disciplines. Such didactic studies as English and history can prove to be "non-liberal" if they are taught in such a way as to disregard the over-all relationship between the particular study and the whole of knowledge. In contrast it would be completely erroneous to say that the natural sciences, for example, had no place in a liberal education. Certainly science has made innumerable contributions to human progress and in doing so has established itself as a cornerstone of liberal education. Scientific knowledge is as necessary to cultural develop-

ment as art, history and philosophy, and with the advance of technology will be held in even higher respect.

To give an example: the plight of the physician is often stated in regard to the need of a broader education. It was Sir William Osler who wrote, "The wider and freer a man's general education, the better practitioner he is likely to be, particularly among the higher classes to whom the reassurance and sympathy of a cultivated gentleman of the type of Eryximachus may mean more than pills or potions. In no other profession does culture count for so much as in medicine, and no man needs it more than the general practitioner, working among all sorts and conditions of men, many of whom are influenced quite as much as by general ability, which they can appreciate, or the learning of which they have no measure."[2] Thus scientific knowledge is a humanistic discipline if it is properly ordered and taught by those whose horizons are wide enough to include the whole universe in view.

It follows that if the liberal arts are directly concerned with making a man truly educated, that each of the academic disciplines identified with the standard departments of our colleges and universities must concern itself with providing the proper atmosphere wherein the student may seek the understanding and appreciation of values and ideas necessary to knowledge and good living. If university departments do not provide this environment, they cannot expect to graduate students who will have the cultural, scientific and social awareness of the kind of world in which they live. However, even a professor is at loss if the student himself does not desire these goals. The university exists to guide the judgment and assist the development of the student and not to indoctrinate a fixed set of attitudes or values.

To bring the problem of the place of the humanistic disciplines into scientific thought we must remember that today our nation is enveloped in an idea of "materialistic vocationalism." This concept will need definition. By "materialistic vocationalism" is meant that our nation in the last half-century, has become completely surrounded or saturated with the importance of the vocations which tend to provide a good living. The rising popularity of the sciences, especially the professional sciences, have added to the widespread idea that a good living is more important than a good life. This misconception can be undermined if one admits the fact that in order to understand Einstein one must first understand Galilio and Heraclitis.

Too often the statement is heard that students frequently make in regard to their professional studies, "Why must I learn the

[2] Severinghaus, A. E., "The Contribution of the Liberal Arts to the Education of the Physician," *Seminar*, Volume XVI, p. 6, Sharp and Dohme, Philadelphia, Pennsylvania, 1954.

humanistic disciplines? They will not help me in my profession." The need for concentration in the basic sciences in preprofessional education can not be denied; nevertheless, too often students who intend to become lawyers, physicians, social workers or business administrators are graduated from colleges and universities with little or no interest in the cultural implications of their professions which later on will enable them not only pattern a good life, as well as a good living, but also provide them with even better means whereby they may render service to their fellowmen.

Moreover the contributions of science in human progress make it necessary to include its study as essential to the cultural heritage of our present day. It holds its own ground along with the contributions of history, philosophy and the social sciences.

The need for the humanistic disciplines in a world of scientific thought has been expressed. But what will be its immediate advantage to society as a whole? What of our nation? How would it benefit by liberal education? The Greeks knew the answer; a democracy could not exist and grow in strength and stature unless it had intelligent citizenry, unless it had an inspired population, unless it had a dedicated people.

An increase of true intellectualism must be stimulated among those of our society capable of expanding their innate faculties to the benefit of mankind. Likewise the "anti-intellectualism" of American thought must be suppressed if world order and peace are to become reality. The plight of the intellectual is indeed desperate. Yet there is hope if intellectualism is once again returned to its noble position in society.

One question seems still virtually untouched. That is, how does one begin to educate himself liberally? Here should be the time when formulas should fly and patterns proliferate. We could speak for hours about schedules and classes, about travel or great books, about how other men have done it. There are countless ways.

But it seems that nearly half the thrill and awe of the liberal arts education is discovering the path for yourself. The definitions come easy, the method will come easier. The only difficult thing is to start it. The howness is basically your problem.

In summary we may say that the ideal of a liberal arts education is a distinct goal which is never reached. Yet it is like a magnet which the closer you get to it . . . the greater the pull. In tasting this wondrous ocean of wisdom, one creates an insatiable thirst. And though our ability to drink this ocean is only as big as a thimble, we cannot help but try to get it all.

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INVESTIGATION METHODS FOR THE EARLY DETECTION OF UTERINE CANCER*

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Although medical science has made such tremendous progresses in the last years, it is still unknown why normal tissue can suddenly grow into a malignant tumour. The success of the treatment of cancer depends in the first instance on the stage of development of the tumour, so it is essential for the final healing to make the diagnosis at the earliest possible moment. It is quite clear that the whole problem of cancer research and treatment concerns the doctors, but in all the modern methods so much laboratory work is involved that also medical technologists have to go further into the matter. Only a well informed staff can guarantee a good result. Of all the cancers in the genital organs of women, the most frequent is the one of the cervix. Fortunately this localization is very adequate for the modern methods of early detection.

So much has been written in the last years about these problems that it seems quite superfluous to add anything, but I would like to point out the procedure which has been developed in the Women's Hospital at the University Medical School, Zurich, because it can give you an idea how these problems are tackled in Switzerland.

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June 1956.

As far back as 1930 the colposcopy, which had been invented by Hinselmann in 1925 in Germany, was introduced in our routine and all patients admitted to the hospital were examined with the colposcop. At the same time the iodine test, inaugurated by Schiller was administered and proved a great help.

The vaginal portio of the cervix is swabbed with an iodine solution and if the epithelium is quite normal, the whole surface colours in dark brown because of the content of glycogen of normal squamous cells. Spots resisting the coloration indicate epithelial lesions, which, of course, are not necessarily malignant. With the aid of the colposcop, a binocular device with a tenfold magnification, these spots can be examined closely. If they prove suspicious, a surface scraping is performed with the sharpened Schillerspoon to secure material for histological examination. Since 1950 we also have had a laboratory for exfoliative cytology which has already gained a very high reputation in the USA. If we compare the results of our colposcopic method with the results of a pure cytology center, as is shown in the following table, we can see that they are almost identical.

Table I

	patients examined	carcinomas found in situ
Colposcopy Zurich 1950-51. Held	7422	40 = 1 : 185
Cytology Montreal Ayre	7830	41 = 1 : 191

So the logical conclusion would be that the results could be twice as good if both methods were used simultaneously. To decide whether the tremendous costs and the work would be worth while, in 1952-53 a series of 7000 women admitted to our hospital were examined by colposcopy as well as by cytology. The two methods were performed separately by different persons so as to obtain absolutely objective results.

Before discussing a few results, I would like to explain the routine methods as we have developed them.

Colposcopy and Schiller's iodine test are always applied simultaneously. In addition, 2400 surface biopsies with the sharpedged Schillerspoon were secured for histologic diagnosis. As a matter of fact, the colposcopy can only yield good results in combination with a large use of histology. If the first histologic examination reveals only an abnormal epithelium, a benign dystrophy, the patient is reexamined 6 or 12 months later. If the epithelium is slightly suspicious of beginning atypical growth, a cervical curettage is performed. If the new histologic diagnosis shows a benign epithelium, the patient is kept in 3-monthly control. If the first examined specimen already shows an atypical epithe-

lium, the cervical curettage is performed at the same time as a ring-biopsy with the cone knife designed by Ayre. The ring-biopsy material is examined in serial sections in order to be able to make a final diagnosis either of carcinoma in situ state 0, or microcarcinoma type Mestwerdt, or already real invasive macrocarcinoma. The therapeutic treatment depends naturally upon this final diagnosis.

May I say just a few words about the handling of the tissue obtained with the sharpedged Schillerspoon.

It is essential that the spoon be really sharpened to obtain a good specimen, that is, a real strip of tissue peeled off the vaginal portio. We have the best results if we fix the material for at least 2 hours in a solution of Bouin's liquid half diluted with 4 percent formalin, and then in alcohol. One has to be careful to put the tissue strips on their edge in the block of paraffin in order to obtain transverse sections. The sections are stained by the usual hematoxilin-eosin counterstain method.

For the cytologic examination we use the original method of Papanicolaou, aspirating vaginal fluid with the curved glass pipette, as well as the method of Ayre, the surface cell biopsy, scraping the cervix in the squamocolumnar area with his especially designed wooden spatula. I must admit that we find a much higher percentage of correct diagnoses with Ayre's method. The smears are stained after the original method of Papanicolaou and examined by specialized laboratory technologists. The doubtful smears, that is, Papanicolaou III and the positive Papanicolaou IV are checked by a cytology specialist.

In our experimental series we found 39 cases of beginning carcinoma, verified by histology, that is, 27 carcinoma in situ stage 0 and 12 microcarcinoma. Among these we had only 4 false negative results within the 27 stage 0 cases and 1 false negative among the microcarcinomas.

The Pap. III although giving an uncertain diagnosis are valuable because they lead to repeated cytological or to histological examination. Our cytological results in the cases of macrocarcinoma are not too satisfactory as we found 4 negatives in 133 cases. Of course, in this category the cytology is not of importance anyway, because these cases would certainly be detected by an ordinary gynecological examination which is generally indicated because of the clinical symptoms. What concerns us now is the detection of precancerous lesions, which we prefer to call "suspicious dysplastic alterations of the epithelium." The colposcopy combined with histology certainly yields better results. Of 43 cases with dysplastic epithelium we found 23 with only Pap. I and II. We cannot say these are all false negatives as it will only be possible by further cytological and histological examinations to find out if these cases develop cancer or not.

In our experience we san say that the cytology is a satisfactory method in the detection of early cancer of the vaginal portio but it is not at all satisfactory for other localisations of cancer. I am not going to speak of cancer of the tubes or ovaries, as they play quite a secondary role, but in the 45 cases of invasive endometrial cancer, we had 16 false negative results and not one of the endometrial cancers were detected by cytology. The cytology, therefore, is of very little value for the early detection of cancer of the uterine body and one should never rely upon a negative result. If ever the clinical symptoms indicate a curettage, this should be performed no matter how many times the cytology gives a negative result.

In the whole series of 7000 patients we have an incidence of false negatives, that is Pap. I or II in cases with histologically verified cancer of 12 percent. False positive results (that is, Pap. IV in cases without cancer) we found in 1-2 percent of the whole series. To check the real value of a method also, the practical side must be taken into consideration. For our series we counted the approximate amount of work involved in both methods.

For the colposcopy we had used about 1150 hours, counting the work of the technical staff as well as the medical. For the cytological work, we needed 2800 hours, far more than double. So the cytology is certainly much more expensive than the colposcopy.

Let us see now what practical conclusions we made out of our experiences: colposcopy as well as cytology proves very efficient for the early detection of cancer of the uterine cervix. Colposcopy is a gynecological method and can only be handled by a specialized doctor. This means that the doctor comes into personal contact with the patient, which is important for the detection of other cancers than the cervical one. Colposcopy, always combined with histology, leads more quickly to a definite diagnosis and is cheaper. Cytology, on the other hand, is the best method to be used outside a gynecological center for routine examination of a large number of "normal" women and proves to be most satisfactory also for control purposes.

A real precancer care center should always be equipped with a colposcopy department, a cytology laboratory and a specialized histopathological laboratory. So both methods can be administered according to necessity, but it is generally not necessary to use both methods in the same case.

Let us hope that we will always find enough funds available and the necessary well trained medical and technical staff in order to let an ever increasing number of women benefit from the latest trends in this field. Only with the earliest possible detection of uterine cancer the healing chance will be approaching 100 percent.

PRESENTATION OF THE 1956 CORNING AWARD

Mr. O. M. Loytty, of Corning Glass Works, makes the presentation of the 1956 Award to Mrs. Lucille Wallace, MT (ASCP), former president of the A.S.M.T.

The presentation speech made by Mr. Loytty follows:

Those who have called our time an Age of Science are to a certain degree in error.

What is closer to the truth is that we are living in an Age of *Applied* Science.

This restriction, this insertion of the adjective "Applied," is an important one. Never before have so many people been so immediately and completely affected by the development and almost instant application of discovered scientific truths.

We do wrong, of course, to sublimate, to underestimate the importance of fundamental research. But because it is fundamental—its direct influence is severely restricted.

It is the technologist—the applier of science—who must translate the discovery into the benefit. To state that in the last decade the ordinary daily chores and pleasures of each of us has been immeasurably changed by the application of scientific truths is no exaggeration.

Unfortunately, the long hours of careful measurement, the sometimes repetitive, sometimes tedious chores of technology lack glamour and excitement for the layman.

Unseen and hardly understood by the outsider is the fact that each benefit he enjoys from science is the result of these daily tasks.

Forgotten by too many is that—but for the men and women who devote themselves to the conversion of fundamentals into practical theories—most scientific observations would rest in the minds of a few men and on the shelves of even fewer libraries.

Despite the preoccupation of newspapers and magazines in recent months with the militant aspects of scientific advancement—no phase of science has so completely hastened progress or contributed so much to mankind mentally, spiritually, as well as physically, as has the field of medicine.

And in no field has the technician played such a dominant, yet unfortunately overlooked role. This group, the medical technologists, the strong left hand of the physician, has taken the theory from the research thesis and brought it into the clinic and laboratory to help, to aid, to improve the general well being of men.

My firm—Corning Glass Works—has its foundations and future firmly anchored on a bedrock of research—both fundamental and applied. Each year, we spend large sums of money in the pursuit of basic knowledge about glass. We thus know perhaps better than many how important it is to have people who can take the information obtained in fundamental research and convert it into everyday applications.

Many of these applications of glass research have been in the medical field. And much of our present good corporate health is a result of the kind attention of medical technologists.

It was this happy association with the field along with our complete appreciation of the work medical technologists are doing that prompted the establishment of the Annual Corning Award to the Year's Outstanding Line Medical Technologist.

This award will consist each year of $500, two pieces of Steuben crystal, and an engraved Steuben crystal presentation bowl.

As you know, Corning Glass Works was not associated with the judging of the nominations. We could not, however, have been more pleased with your choice for the 1956 award. Many of you know the recipient and all of you, I am sure, are familiar with her work. It would be presumptious of me to attempt to review her outstanding career. It is surely sufficient to agree with the judges that no one more fittingly represents the profession of medical technology than she does.

It thus gives me great pleasure to present the Corning Award for 1956 to Mrs. Ida Lucille Brown Wallace.

PHOTOGRAPHY CONTEST 1956 AWARD WINNERS

Sponsored by the Public Relations Committee ASMT

FIRST PRIZE: Mildred E. Olson, Minneapolis, Minnesota

SECOND PRIZE: Mrs. Merle Rickly, Memphis, Tennessee

THIRD PRIZE: Mrs. Mary K. Cressman, Evanston, Illinois

HONORABLE MENTION

Left: by Miss Nellie May Bering, Washington, D. C.

Center: by Miss Marianne Schaaf, Milwaukee, Wisconsin

Lower Left: by Miss Anna Bell Ham, Coral Gables, Florida

Lower Right: by Miss Audrey Alteimatt, Milwaukee, Wisconsin